

LIFE HISTORY STUDIES ON TWO FROG LUNG FLUKES,
PNEUMONOECS MEDIOPLEXUS AND *PNEUMOBITES*
PARVIPLEXUS

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INTRODUCTION

In spite of the fact that frogs are commonly used the world over as subjects for biological examination and experimentation, trematodes which live in this amphibian are still being discovered. This is noteworthy when one considers that a trematode parasite was observed in the frog as early as 1737. *Distomum cylindraceum* was not only the first to be recorded from frogs, but has the distinction of being one of the earliest known trematodes. No doubt this parasite and others have been seen by innumerable individuals since that time. Why the life history of more of these forms has not been the subject of inquiry is perplexing.

The present paper deals with the life history of two frog lung flukes, *Pneumonoeces medioplexus* Stafford and *Pneumobites parviplexus* (Irwin), a problem which was chosen while a student at the University of Michigan Biological Station in 1927 and pursued for several summers thereafter. During the

* Contribution from the Zoölogical Laboratory and the Biological Station of the University of Michigan.

winters the research was continued at the University of Michigan. At the time the problem was begun little was known of the development of frog lung flukes. For the genera *Pneumonoeces* and *Pneumobites* only the second intermediate host of one European form was known.

In this work the hosts of *Pneumonoeces medioplexus* were found to be the snail, *Planorbula armigera* (Say), dragonflies of the genus *Sympetrum*, the frog, *Rana pipiens* Schreber and the toad, *Bufo americanus* Holbrook; while the hosts of *Pneumobites parviplexus* (Irwin) are the snails, *Gyraulus parvus* (Say), dragonflies of the genus *Sympetrum*, and the frog, *Rana clamitans* Latr.

In the final experiments for life history determination all hosts except the green frog and the common toad were raised from eggs and experimentally infected in the laboratory. The green frogs were collected as tadpoles from an acid lake and kept in aquaria until transformation. Most of the snails for the experiments were collected from Fontinalis Run, a swamp near the University of Michigan Biological Station on Douglas Lake in Cheboygan County, Michigan, and from two pools near Ann Arbor, Michigan. For the preliminary experiments dragonfly nymphs and leopard and green frog tadpoles were collected from Vincent Lake, a small acid lake free from snails, near the Biological Station. Supplementary material was taken from Third Sister Lake, near Ann Arbor, Michigan.

A preliminary report briefly describing these two life histories has been published in the *Journal of Parasitology* (Krull, 1930).

I wish to take this opportunity to express my appreciation to Dr. George R. La Rue, under whose direction these studies were made, for his interest, helpful suggestions and criticisms. To Dr. W. W. Cort of Johns Hopkins University, I am indebted for helpful suggestions and the loan of his lung fluke material used in the verification of species, and to Dr. W. A. Riley, University of Minnesota, for the loan of a mounted specimen of *Pneumobites parviplexus*. I am grateful to Mr. Calvin Goodrich and Mr. E. B. Williamson of the Museum of Zoölogy, University of Michigan and to Dr. C. F. Byers of the University of Florida for the identification of hosts.

HISTORICAL ACCOUNT OF ADULTS

Johnston (1912) gave a historical review of the frog parasites, and Cort (1915a) gave an account of the frog lung flukes. According to Johnston, Swammerdam (1737) was probably the first to record the observation of a trematode living in the frog, which is now known as *Haplometra cylindracea*. It is not only the first trematode obtained from frogs but is one of the oldest known. Pallas (1760) mentioned a *Fasciola subclavata ore sessili* in the lung of the frog, in the intestine of the pike, and in the liver of sheep. Goeze (1782) also recognized a lung fluke in the frog and called it *Planaria cylindrica*; and Zeder (1800) described and named it *Distomum cylindraceum*. Looss (1899) created

the genus *Haplometra* for this worm. Its life history to some extent was worked out by von Linstow (1890).

Apparently the first species of *Pneumonoeces* to be described was *P. variegatus*. It was known to Zeder who, because he failed to see the ventral sucker, placed it with *Monostoma* and described it as *Monostoma bombynae*. Rudolphi (1809) at first also failed to see the ventral sucker but later (1819) he recognized it and described this lung fluke from *Rana esculenta* as *Distoma variegatum*. Looss (1894) gave it an adequate description and later (1899) separated his *Distomum variegatum* material into three distinct species for which he created a separate genus, giving the names *Haematoloechus variegatus*, *H. similis*, and *H. asper* sp. inq., to the three species. Later Looss (1902) changed the generic name *Haematoloechus* to *Pneumonoeces* on account of the hemipteron genus *Haematoloecha* established in 1874.

In America Leidy (1851) was the first to record lung flukes from frogs but his descriptions are too brief to be of any diagnostic value. In 1902 Stafford described five species under the names *Haematoloechus longiplexus*, *H. breviplexus*, *H. similiplexus*, *H. medioplexus*, and *H. varioplexus*, and in a later publication (1905) designated them as belonging to the genus *Pneumonoeces*. Pratt (1903) described *Ostiolum formosum* which Stafford (1905) and Cort later (1915a) considered to be a synonym of *Pneumonoeces medioplexus*. In 1906 Seeley added *P. complexus* to the list. Cort (1915a) redescribed some of Stafford's species, Seeley's *P. complexus*, and added the description of *P. coloradensis* as new. Because of the incomplete description of *P. varioplexus* and the fact that no specimens of it could be obtained for examination from Stafford, Cort (1915a) was obliged to treat it as a species inquirenda. Irwin (1929) described *P. parviplexus* which is the last of seven species of lung flukes described from North American frogs.

In Table I are listed the North American species with the hosts and regions from which they were taken. From Table I it will be seen that six of the seven species have been taken from *Rana pipiens* (*R. virescens*), two from *R. clamitans*, two from *R. catesbeiana* and three from *Bufo americanus* (*Bufo lentiginosus*).

The genera *Pneumonoeces* and *Pneumobites* belong in the family Plagiorchiidae Lühe (1901) concerning which much confusion has arisen because the genera *Plagiorchis* Lühe and *Lepoderma* Looss appeared in journals printed apparently on the same day, but the distribution of the journal containing Looss's paper was delayed. Stiles (1901) accepted the name Plagiorchiidae for the family and Poche (1926) gives a detailed account of the difficulties involved in the synonymy of these names and concluded that the name *Plagiorchis* had priority and consequently the family name must be Plagiorchiidae. This family is one of the largest and most heterogeneous of the trematodes and frequent attempts have been made to subdivide it into subfamilies. Poche

TABLE I. HOSTS AND GEOGRAPHIC DISTRIBUTION OF FROG LUNG FLUKES IN NORTH AMERICA

Parasite	Hosts	Locality	Authority
<i>Pneumobites longiplexus</i> (Stafford)	<i>Rana pipiens</i> <i>R. catesbeiana</i>	Chicago, Illinois Urbana, Illinois North Judson, Indiana Ontario Quebec New Brunswick Nova Scotia	Cort (1915) Stafford (1902)
<i>P. breviplexus</i> (Stafford)	<i>R. clamitans</i> <i>Bufo americanus</i> <i>R. catesbeiana</i> <i>R. pipiens</i> (<i>R. virescens</i>)	North Judson, Indiana Oklahoma North Judson, Indiana Canada Canada	Cort (1915) Stafford (1902)
<i>P. parviplexus</i> (Irwin)	<i>R. clamitans</i>	Litchfield, Minnesota Ann Arbor, Michigan Douglas Lake, Michigan	Irwin (1929) Krull (present paper)
<i>Pneumonoeces medioplexus</i> Stafford	<i>R. pipiens</i> <i>R. pipiens</i> (<i>R. virescens</i>) <i>B. americanus</i> (<i>B. lentiginosus</i>)	North Judson, Indiana Ann Arbor, Michigan Douglas Lake, Michigan Canada Canada Douglas Lake, Michigan	Cort (1915) Krull (present paper) Fortner (1923) and Krull (present paper) Stafford (1902) Krull (present paper) (experimental)
<i>P. similiplexus</i> Stafford	<i>R. pipiens</i> <i>R. pipiens</i> (<i>R. virescens</i>) <i>B. americanus</i> (<i>B. lentiginosus</i>)	Douglas Lake, Michigan Canada Canada	Cort (1915) and Fortner (1923) Stafford (1902)
<i>P. complexus</i> Seeley	<i>R. pipiens</i>	North Carolina	Seeley (1906) and Cort (1915)
<i>P. coloradensis</i> Cort	<i>R. pipiens</i>	Boulder, Colorado Colorado Springs, Colorado	Cort (1915)

was the last to give a synopsis and rearrangement of the genera belonging in the family.

According to the elaborate classification by Poche the frog lung flukes belong in the Order Digenea, Suborder Prosostomata, Tribe Fascioloidae, Subtribe Fasciolinae, Supersuper-family Fasciolida, Family Plagiorchiidae in which he places 35 genera. Baer (1924), who included 32 genera in his classification of the Lepodermatidae (Plagiorchiidae), distinguished five subfamilies, namely: Saphedratinae Baer, Lepodermatinae Looss e.p. Baer, Cymatocarpinae Baer, Brachycoeliinae Looss, and Astiotrematinae Baer.

Baer's diagnosis of the subfamily Saphedratinae, to which belong the two species that form the subject of the present paper, reads: "Lepodermatidae, found in lungs of Amphibia and Reptiles. Characterized by the position of the genital pore which opens at the base of the pharynx, cirrus pouch very long and thin. Cuticle may be beset with spines. Lauer's canal absent in the genus *Pneumonoeces*. Testes parallel, one slightly in front of the other. *Saphedra* has testes in the tandem position. Yolk-glands scanty in grape-like formation. To the Saphedratinae belong *Saphedra* Looss, *Pneumonoeces* Looss, and *Pneumobites* Ward."

Recent study shows that Lauer's canal is absent in *Pneumobites* as well as *Pneumonoeces*, and the diagnosis of the subfamily should be changed accordingly. Ward (1917) created the genus *Pneumobites* for *Pneumonoeces longiplexus* Stafford (type of genus) and *P. breviplexus* Stafford and in Ward and Whipple (1918) he modified the original characterization to read: "Much like *Pneumonoeces* but body larger, thicker, with testes lobed, elongate, lateral and symmetrical or only slightly oblique. Extracecal longitudinal folds of uterus pronouncedly longer than in *Pneumonoeces*. Ovary lobed. Vitellaria with many very small acini in each group. Eggs small." Irwin who described *Pneumonoeces parviplexus* apparently was not aware of the genus *Pneumobites* since references by Cort (1915a) and Stafford (1902) are the only ones included in her bibliography. *Pneumonoeces parviplexus* rightly belongs in the genus *Pneumobites*.

GENERAL METHODS AND TECHNIQUE

The general methods and technique involved in these life history studies are discussed in this section. More detailed methods used in the work are found in the parts dealing with the biology of the parasites and with the description of the life history stages.

In determining trematode life histories it is necessary, if the best results are to be obtained, to make a general survey of the region in which the work is to be done. In this way information is acquired on the abundance and general distribution of the parasite to be studied. After such a survey has been completed one saves time by concentrating on the best locality. It is possible to exhaust the supply of material in a given place and if a survey has been made no time is lost in case the stock becomes depleted. Certain factors, some of

which have been mentioned, should be borne in mind when selecting a place as a source for material. A small fauna is often desirable. This is especially true for snails, since the presence of only a few species not only reduces the number of possible kinds of cercariae to be found but it also saves time. The permanence of conditions during the summer should influence the choice of locality, for often conditions change decidedly during the summer, as well as from one season to the next, so that work may actually have to be discontinued. Possible interference and destruction of natural conditions by disinterested persons must also be considered.

Since it was known that trematodes in closely related genera used insects as second intermediate hosts it was assumed that insects would be the second intermediate hosts of the lung flukes. Insects of all kinds were examined for metacercariae and when specimens were found that were suspected of being metacercariae of frog lung flukes they were fed to frogs which were examined later. Stomach contents of frogs were also examined to determine what they were taking as food. Snails, first intermediate hosts of *Pneumonoeces* and *Pneumobites* were found only after the possibilities of finding cercariae in the larger snails had been exhausted and the technique of examining the water in the bottles containing snails had been changed. The fact that several different kinds of cercariae penetrated into dragonfly nymphs was very misleading.

These life histories were discovered in nature by beginning with the flukes in the final host and working backward. The metacercariae were found in the insect and lastly the larval forms were found in the snail. When the hosts had been determined, cercariae escaping from the snail were necessarily used as the starting point in the laboratory infections until the methods of hatching the parasite's eggs and of rearing and infecting snails were discovered. After these methods had been worked out the snails to be experimentally infected were secured by hatching eggs deposited by snails taken from ponds. Then the study of the life history of the fluke was begun with the feeding of the parasite's eggs to laboratory-raised snails.

The naturally infected molluscs used in this life history work were collected with decaying vegetation from ponds and placed in a bucket and left over night. The snails came to the top and were picked off the following morning with a pair of forceps. *Planorbula armigera* selected for breeding purposes were washed thoroughly on the day they were collected. They were then transferred to suitable containers, fed on food known to be free from parasite eggs, and washed each day for a fortnight. This precaution was taken to avoid the possibility of contamination caused by parasite eggs sticking to the snail and its shell. *Gyraulus parvus*, used for the securing of eggs, were kept in the laboratory all winter (Krull, 1931).

The dragonfly nymphs used in this study were taken from two sources. In the early part of the work they were collected from Vincent Lake, a small acid lake, in which snails do not occur and from which infected dragonflies have

never been taken. The nymphs were collected with a dredge net. Later others were raised from eggs in fruit jars and fed on food reared in the laboratory (Krull, 1929).

The cercariae used in infecting nymphs were isolated from the snail and counted before they were placed in water with the nymphs. Sometimes when cercariae from *P. armigera* were used the nymph was placed in the vial with the snail and cercariae. This could not be done with the smaller and more delicate *G. parvus* because the nymph ate the soft parts of the snail.

All frogs of special significance in this study, except the one used in the final experiment for the life history determination of *Pneumonoeces medioplexus*, were collected as tadpoles in the same lake from which the nymphs were taken. The tadpoles until transformation were kept in small aquaria and fed on decaying lettuce. After transformation the frogs were kept in small, solid-walled pens covered with a netting having a mesh large enough to admit flies. Some small pens consisted of boxes with solid sides and covered with wire netting of the same sized mesh. Other pens, four feet square, were made by covering wooden frames with canvas. Neither type of pen had a bottom so each was buried in the ground several inches and fastened down. As many as 40 or 50 frogs could be kept in each of the larger pens whereas 20 could live in the smaller ones. Every pen was provided with water by fitting in a shallow pan so that the top was level with the ground. All frogs in the summer and small frogs in the winter were fed more or less automatically. During the summer, pieces of dead fish or dead clams were placed in the pen where there was sunshine. The decaying flesh attracted numerous flies which the frogs caught. By this means the frogs were always well fed, were fat and grew faster than those in nature. The amount of dead material in the pen must of necessity be small to prevent the production of large numbers of maggots for these, when eaten in quantity, are fatal to frogs. In the winter decaying lettuce was kept in the aquarium room near the netting-covered vessel containing small frogs. Fruit flies breeding in the decaying lettuce were attracted to the frogs by placing pieces of apple in the containers. Large frogs were occasionally fed earthworms during the winter. These worms were collected late in the fall and kept in boxes of moist earth or in moist chambers containing a few dead leaves and a mat of soil and moss the size of the dish. The latter was kept well watered and covered with a glass plate.

Frogs in covered and uncovered pens were kept near the experimental pens during the summer as a check against possible contamination. These frogs were examined in the fall and findings were all negative. In this vicinity there were no breeding places for *Sympetrum*; however adult dragonflies were observed on three occasions. Two were caught and examined but both were negative for larval flukes.

The life history of *Pneumonoeces medioplexus* as determined under laboratory conditions, is based on ten preliminary experiments and a final experiment.

That of *Pneumobites parviplexus* is based on five preliminary experiments besides the final.

A typical preliminary experiment dealing with *P. medioplexus* is here described. Thirteen dragonfly nymphs which had been infected with the cercariae produced by one infected snail were retained until their metacercariae were old enough to be infective. Some were then dissected from the nymphs and preserved for study, others were given to ten leopard and four green frogs. Since the dragonfly species involved as hosts are common for the two species of flukes the green frogs in this experiment were used as controls to determine the possibility of a double infection in the snail. The leopard frogs were examined one by one so that a series of developing flukes was secured. During the experiment the snail and nymphs were marked by labeling the containers in which they were kept. The frogs were marked by cutting off different combinations of toes. In each experiment with *P. parviplexus* fewer hosts were involved than in *P. medioplexus*.

In the preservation of life history stages cercariae were killed by plunging them into hot 5 per cent formalin, and were preserved in a solution of the same strength. Two methods were used for metacercariae. Some were preserved in corrosive-acetic fixative from saline solution, others were placed in cold water until they relaxed before fixing in corrosive-acetic. After fixation they were treated with iodine, dehydrated as usual and preserved in 70 per cent alcohol. Juvenile and adult worms were treated the same as the metacercariae. When adults with many eggs were relaxed they were left in cold water until most of the eggs had been shed. It takes from 10 to 60 minutes depending on the number of eggs and the size of the fluke. This treatment causes no serious changes in structure, except that parts of the cuticle slough off. If there is only a slight lobing of the sex organs this method may destroy the irregularities because of absorption of water but any pronounced lobing is not affected. Well-extended worms, in which the organs are most natural, were secured by taking them from normal saline and plunging them into hot 70 per cent alcohol. Specimens fixed in this way do not stain as well as those treated with corrosive-acetic. For whole mounts of all stages, a stain made from wild grapes was used with excellent results and the flukes after staining were cleared in synthetic wintergreen oil and mounted in damar or balsam. Iron haematoxylin and eosin were used in staining sections.

Snails for sectioning were fixed in Bouin's solution and stained with haematoxylin and eosin. Dragonfly material for sectioning was fixed in Carl's fluid and then handled according to the methods of Tillyard (1917).

BIOLOGY OF PNEUMONOECS MEDIOPLEXUS STAFFORD

Experiments with Adults and Juveniles. *Pneumonoecs medioplexus* is a common parasite in the lungs of the leopard frog, *Rana pipiens* Schreber, and has larval stages in the snail, *Planorbula armigera* (Say), and certain drag-

on flies of the genus *Sympetrum*, the metacercariae having been raised under controlled conditions in *S. obtrusum* (Hagen) and *S. rubicundulum* (Say). This fluke has been taken from the common toad, *Bufo americanus* Holbrook, by Stafford (1902), who also took it from *R. virescens* Kalm., a synonym of *R. pipiens*. I have tried to infect green frogs experimentally, using a total of 16 specimens, and in no case did the parasite reach maturity. These frogs were given generous doses of metacercariae and the longest that *P. medioplexus* lived in this species of frog was 14 days, and in each of two cases a single immature fluke was found at the end of that time. Seven frogs examined after the fourteenth day were negative. During the time that these parasites remain in this species of frog the trematodes apparently grow normally and it may be possible that they occasionally reach maturity. *P. medioplexus* has not been reported from the wood frog, *R. cantabrigensis*. Wood frogs from regions where leopard frogs were heavily infested with this lung fluke have always been negative when examined. In trying to infect this species experimentally 16 small wood frogs (25 mm.), raised in the laboratory from eggs, were each given a generous number of metacercariae that had been raised under controlled conditions. The frogs were examined 39 days later and all were negative.

Numerous tadpoles of *R. pipiens*, taken from a swamp where there were many dragonfly nymphs with heavy infestations of metacercariae, have been examined but lung flukes have never been recovered. Findings in nature and my experimental results with *Pneumobites parviplexus* of the green frog, the other form discussed in this paper, seem to indicate that tadpoles never become infected with lung flukes.

The percentage of frogs infected in a given locality is in a large degree dependent upon the presence of the intermediate hosts since both snails and dragonflies are necessary for the perpetuation of the species. In the Ann Arbor region 11 out of 53 frogs were infected while in the Douglas Lake region 59 out of 106 were parasitized. Large frogs are more apt to be infected than small ones. If frogs are separated into two groups, those with a body length of 45 mm. or less and those 46 mm. or more, the percentage of infection is from 20 to 25 per cent greater in the large frogs. This condition appears to be due to two factors. There is much difference in the size of young leopard frogs at the time of transformation. Some of the small frogs just after metamorphosis have difficulty in eating the adult dragonflies, but the larger ones do not. After creeping up to the edge of a swamp on a hot summer afternoon I dropped injured dragonflies close to small leopard frogs and watched while the insects were devoured. Several small frogs had difficulty in swallowing the old, well-chitinized insects, while somewhat larger frogs had no difficulty. The other factor affecting the percentage of infection is the distribution of frogs according to size and age. Small, i.e., young, frogs, as a rule, spend practically all of their time close to the pool or on vegetation mats in the pond, and consequently do not have as good an opportunity of securing the dragonflies as do the large

old frogs. The sedges are usually high and thick around the pond or in the swamp. The dragonflies do not penetrate the vegetation but fly above it and as a rule alight only on the upper parts. This is high enough to be out of the range of the frogs; however, small ones have actually been seen trying to catch these insects. Apparently the smaller frogs obtain most of their infections by catching the soft tenerals which in the morning hang to the stems above the mats or close to the edge of the pool. They may take the nymphs as they emerge from the water for it has been observed that frogs will eat the nymphs, although they refuse some of the other insects which normally live in the water. Water to a depth of two inches was put into a large, clean moist chamber, and before being covered with a glass plate dragonfly nymphs and a leopard frog were introduced. After several days the nymphs were gone. This was repeated with the green frog with similar results. Both kinds of frogs also will eat dragonfly nymphs which are out of the water. The larger frogs do not confine themselves to the region around the pool but go into surrounding grass lands to catch insects. At dusk the dragonflies also seek similar situations. They alight on the grass stems or small shrubs, if they are present, and crawl down these stems near to the ground probably to protect themselves from the cold and the dew of the night. This brings them into the range of the frogs which catch them at this time and thus secure an infection. This, perhaps, explains why one rarely finds the remains of dragonflies in the stomach contents of frogs; for by morning the material has usually been too completely digested for recognition.

The host-parasite relationship between the frog and the lung fluke seems to be faultless. It is impossible to kill a frog by an overdose of metacercariae because only a certain number of them will live and grow in the lungs. Frogs were fed numerous infected dragonflies and about 24 to 36 hours later an examination of the lungs revealed more than a thousand larval flukes in each frog. Some of the frogs which were given an abundance of metacercariae were kept on a table for observation and it was noticed that occasionally they opened their mouths and made a hissing sound, apparently by allowing the air to escape quickly from the cramped lungs through the glottis which was only slightly opened. Observations of control frogs showed that they also expelled the air by force but not so often as did the infected frogs while the metacercariae were migrating.

In order to learn something about the activity of the metacercariae after entering the final host a leopard frog was pithed and the digestive tract immediately laid open as far as the intestine without removing any part. Then a number of cysts in a small amount of water was placed in the stomach. The parasites immediately responded by becoming active and escaping from the cysts. The anterior parts of the stomach and esophagus of the frog were somewhat folded longitudinally and streams of mucus were rapidly carried from the

mouth to the stomach by ciliary activity of the epithelial cells. When freed from the cysts the metacercariae began to travel toward the mouth against the mucous currents by the use of their suckers and the active contractions of the body. Locomotion was assisted materially by the small retrorse spines on their bodies. When they came to the folds of the stomach and esophagus they worked their way forward between them. By the time the larvae were about half way up the esophagus the tissues were too dry for them to make any further progress. A drop of normal saline was added and the parasites completely changed the direction of their movement. The response therefore seems to be chemotactic.

In another experiment a medium-sized leopard frog which had been kept in the laboratory for one month was selected. This period of time was sufficient to permit any parasites in the lungs to mature and become large enough to prevent confusing them with parasites a day old. After this period of isolation the frog was given the abdomens of 300 adult *Sympetrum*. Twenty-four hours after feeding; the mouth, esophagus, stomach, larynx, and lungs were taken from the frog and quickly isolated from one another and examined in saline. A small number of larval trematodes was found in the stomach along with some of the chitinous insect parts. Twenty-two metacercariae were recovered from the esophagus. Those in the posterior end were actively migrating while those in the anterior part seemed to be content, many had taken blood, the ceca being full. One was found on the roof of the mouth, seven on the tongue, but none underneath the tongue. Four, whose digestive systems were full of blood, were found in the larynx. The majority, 690, were in the lungs and many of these had taken blood. This experiment shows that the larvae may feed while they are migrating and that the majority complete their migration within 24 hours.

For the experimental infection of frogs warm weather is very desirable. Fairly good infections have been obtained with temperatures as low as 60°F., but below that results are decidedly erratic.

Frogs may be given metacercariae in different ways. The insect, adult or nymph, may be fed entire or the cysts may be removed and fed with a dropper. The metacercariae, free from the cyst, in saline or water are equally infective. The freed or encysted larvae when inserted directly into the glottis are infective, since the cysts are very delicate and open readily as will be pointed out later.

An experiment was carried out with small leopard frogs (40-45 mm.) to determine the number of parasites which could be recovered when frogs are given a comparatively small known number of metacercariae. The metacercariae were taken from laboratory-infected nymphs and given to frogs which were then kept under ideal conditions for a sufficient period of time to allow most of the flukes to become mature. The results of the experiment are given in Table II from which it may be seen that when comparatively small numbers

TABLE II. SHOWING RESULTS OF FEEDING FROGS A KNOWN NUMBER OF CYSTS

Host Number	Days elapsed after infection	Number of cysts fed	Number of parasites recovered	Percentage recovered
1	49	12	10	83
2	49	12	1*	8
3	49	12	12	100
4	221	12	1	8
5	29	14	10*	70
6	29	14	5	36
7	65	14	7	50
8	65	14	8	57
9	65	14	0	0
10	65	14	12	86
11	100	14	2	14
		Total 146	Total 68	Average 46

* Flukes immature.

of metacercariae are fed approximately half of them live and become mature.

The usual number of mature flukes is not large in naturally-infected frogs. There are exceptional cases, however, in which the bulk of mature parasites in the lungs is greater than the bulk of the lung tissue. From Oshkosh, Wisconsin, Cort (1915a) reported the heaviest infection of leopard frogs in which 42 full-grown specimens were found. Fortner (1923) who studied the green, leopard, and wood frogs, reporting his heaviest infections as occurring in the leopard frog, took 72 flukes from a single host, but unfortunately did not state whether these were mature. My largest number (74) of mature specimens were taken from a frog (61 mm.) which was collected on June 27. In frogs infected in the laboratory with metacercariae from experimentally infected dragonflies the largest number of mature flukes was taken from a frog 40 mm. long. From this frog, infected 35 days, 27 flukes were recovered. On account of lack of material, no attempt at overdosing was tried with experimentally raised metacercariae, however, each of a number of small parasite-free frogs was fed several thousand metacercariae which were collected from dragonflies in nature. These frogs were then kept under controlled conditions to determine the outcome. One 42 mm. frog which died from red leg 36 days after being infected yielded 82 *Pneumonoeces medioplexus* most of which were mature but all small. The other frogs survived. Two of them were kept as controls from August 14, 1928, to August 28, 1929. These frogs became fat and appeared healthy. When examined one frog (58 mm. long) was negative for lung flukes, the other (63 mm. long) had one mature fluke which was rather opaque and showed what were thought to be degenerate changes, perhaps of old age.

Mature specimens of *P. medioplexus* vary greatly in size and this is influenced somewhat by the technique of handling them after being removed from the lungs. Stafford (1902) stated that his longest mounted preparation was 11 mm., but that living ones may measure as much as 15 or 16 mm. One of Cort's (1915a) largest mounts (7.8 mm.) was killed in saturated corrosive sublimate plus 1 to 3 per cent acetic acid. My largest mounts, killed in corrosive-acetic, and obtained from naturally infected frogs from Michigan measure not over 8.5 mm. in length. In one experiment a number of naturally infected frogs, kept over a year under controlled conditions, yielded several specimens 10 mm. long. The longest fluke of this species in my collection is 11 mm. It was taken from a leopard frog collected in North Judson, Indiana. Of my experimental material, 95 mature parasites ranging in age from 24 to 320 days, were measured, the largest being 8.75 mm. in length and 109 days old.

These worms mature when very small. Cort's (1915a) 3.9 mm. specimen was the shortest on record up to that time. Sixty flukes reared by me under controlled conditions from the cercarial stage, ranged in length from 1 to 3.75 mm. Of these 18 were mature and 42 immature. They were taken from 10 frogs from six different preliminary experiments.* The age of the flukes ranged from 14 to 65 days. No parasite less than 2 mm. was mature and none more than 3.5 mm. was immature. The shortest mature specimen was 2.25 mm. and was one of two flukes found in a 46 mm. frog. The youngest mature example was 24 days old and was one of 15 in a 40 mm. frog. The longest immature fluke was 3.5 mm. long, 24 days old and was one of 15 specimens in a 40 mm. frog. Twenty flukes 16 days old were immature. Of 21 examples 24 days old nine were mature and 12 immature. These nine flukes had few eggs in the uterus so that 24 days may be considered the shortest period at which they mature. Of nine flukes 29 days old three were mature. Fifteen 30-day-old specimens were examined and five were mature. Fifteen flukes 35 days old (from two frogs) were mature and each contained numerous eggs. The majority of worms become mature in 28 to 30 days and after 35 days all parasites are usually mature but some are very small.

Table III gives the lengths of flukes at various ages. For measurements all flukes were put into cold water until relaxed; then killed in corrosive-acetic fixative, and stained and mounted.

Table III shows that there is a uniform increase in the length of the flukes up to the time of maturity, after which other factors have to be considered to explain the peculiarities of size. The data indicate that the number of parasites in a frog and its size are of secondary importance. These variations in the size of the parasites seem to be due to internal conditions of frogs *inter se*. That frogs differ in the conditions which they present for parasites can hardly be

* Described in the section on methods and technique.

TABLE III. SHOWING LENGTHS OF FLUKES IN RELATION TO AGE

Number of flukes	Age in days	Range of lengths in mm.	Average length in mm.
9	12	0.40-0.85	0.64
6	14	0.50-1.25	0.87
16	16	0.75-1.25	1.11
17	24	2.00-4.25	2.89
9	29	1.25-3.25	2.25
15	30	1.75-3.25	2.50
11	35	3.00-5.00	4.22
5	41	4.50-5.50	4.95
5	49	5.25-6.75	6.18
2	63	5.75-6.00	5.87
3	64	2.25-5.50	4.25
22	65	3.75-7.25	5.61
8	90	4.00-5.75	5.00
5	98	5.75-7.00	6.35
4	109	7.75-8.75	8.31
4	115	4.75-5.75	5.31
10	117	6.00-7.00	6.60
8	259	5.75-6.50	6.00
159 Total			

denied. These conclusions were supported by the following observations and experiments.

A leopard frog from each of two preliminary experiments was examined on November 30, 1930. One 41 mm. frog at 109 days after infection had six lung flukes markedly uniform in size and varying in length from 7.75 mm. to 8.75 mm., average 8.3 mm. The other frog, 38 mm. long at 115 days after infection had seven flukes varying in length from 4.75 mm. to 5.75 mm., average 5.3 mm. In comparing these frogs and their parasites it can be said that the larger frog had much larger flukes than the smaller frog although the former's flukes were six days younger. Similar results were secured repeatedly. The difference in number of parasites (one fluke) cannot account for the variation in size as a number of similar cases show, one of which will be described.

A leopard frog 48 mm. long was fed 12 cysts and kept for 49 days. When examined 10 flukes were recovered ranging in length from 5.25 to 6.75 mm., average 6.2 mm. The other frog, 38 mm. long, was given not over a dozen metacercariae and when examined 115 days later 7 flukes were recovered ranging in length from 4.75 mm. to 5.75 mm., average 5.3 mm. In this illustration it is shown that the frog kept for the shorter period of time had more and larger flukes. In my experience with experimental infections there is no correlation in the first-year frogs between the size of the host and the size of the

parasite. It is just as probable that in very small frogs the mature parasites will be as long as those in larger frogs.

Concerning longevity and size of infestations, *Pneumonoeces medioplexus* may live in the frog for a little more than a year. Frogs become infected one summer, and keep the flukes through the following fall and winter. The parasites are apparently discarded one by one during the following summer and fall, while the frog is acquiring and maturing a new infection. These statements are supported by the results of the following experiment.

On July 22, 1928, each of several medium-sized frogs was given a large number of metacercariae obtained from naturally-infected dragonflies collected from the same region and at the same time as the frogs. The frogs were given these cysts to make sure that they carried an infection of lung flukes. By experimental means it had been found previously that the majority of metacercariae in the naturally-infected dragonflies of the locality belonged to this species (*P. medioplexus*). The control frogs were kept away from the possibility of natural infections for a period of nearly a year and a half during which they were killed one at a time at varying intervals, as shown in Table IV.

The longest time that flukes were retained by the frogs in this experiment was 15 months. It is true that there are not enough frogs in the experiment to warrant a definite conclusion in regard to the length of time that the flukes are retained by the frogs, but data of this nature are difficult to obtain and

TABLE IV. SHOWING RESULTS OF EXAMINATION OF FROGS INFECTED
JULY 22, 1928

Specimen Number	Length in mm.	Date of Examination	Elapsed time in days	Lung flukes found
1	60	VII-25-1928	3	29 immature
2	60	VII-30-1928	8	7* immature
3		VIII- 9-1928	18	21 immature and 2** large mature
4	77	IX- 6-1928	46	35 mostly mature
5		XII-18-1928	149	14 large mature
6		II-18-1929	211	12 large mature
7	56	IV-19-1929	271	1 large mature
8	64	VII-19-1929	362	2 large mature
9	61	VII-19-1929	362	6 large mature
10	65	X-15-1929	450	3 large mature
11	68	XII-11-1929	507	Negative
12	65	XII-11-1929	507	Negative

* Forty-eight mature roundworms in the lungs may have been the cause for the small number of flukes.

** Held over from an earlier infection.

it is hoped that in the future more information may be gained in support of the findings here given. The experiment shows, however, that the number of parasites in the host is decidedly reduced as the period of infection increases beyond seven or eight months.

Two leopard frogs from another experiment gave additional information concerning the length of life of the parasite in the frog. These frogs were collected as tadpoles in Vincent Lake during the summer of 1928 and after transformation they were each given 14 experimentally raised metacercariae on August 14, 1928, and were examined on August 28, 1929. One frog was negative and the other had one fluke. It appears that the life span of this parasite in the frog is about a year.

Parasites collected from frogs in nature have occasionally appeared to be abnormal and the conditions observed suggest signs of old age. A fluke from specimen No. 9 of Table IV and the single parasite mentioned in the last paragraph showed distinct abnormalities. When removed from the lungs these flukes act normally, but they appear more transparent and glossy. The ovary is large, yellowish, and more opaque than normal; the testes are small and indistinct. The uterus when full of eggs is a dirty, light brown color instead of the characteristic dark brown. This change, no doubt, is the result of the numerous abnormally small and imperfectly formed eggs in the uterus. In one specimen the terminal part of the uterus was empty. These abnormal flukes do not stain properly and the differences resemble those that one finds as a result of the poor staining which follows poor fixation.

The physical condition of the frog apparently does not noticeably affect the growth of the parasite nor does it affect the number of flukes which reach maturity and remain in the lungs for any length of time. This was found to be true of frogs, whatever the cause of emaciation, whether it be forced starvation, injury of the jaws which prevented feeding, or refusal to eat. The following extreme case verifies the above conclusion that emaciation does not affect the growth of the parasite. Several frogs which had been kept in an outdoor pen were moved in the fall to an aquarium indoors and kept at a temperature of about 60°F. During the confinement indoors one of the frogs became more and more emaciated until finally it was too weak even to jump normally. It was too feeble to take food and was kept alive for a month after this time by forced-feeding with pieces of earthworm every few days. When examined, November 19, 1929, the frog, only 30 mm. long, had five flukes in the lungs (98 days after infection). Their average length was 6.35 mm., exceeding in size older flukes found in larger and better fed frogs that carried fewer parasites in the lungs.

There is no good reason for thinking that lung flukes cause emaciation. The frogs with the heaviest infections appeared normal. The flukes, however, have an effect on the lung tissue, causing it to be somewhat thicker and less

spongy than in normal lungs. In parasite-free frogs the lungs are spongy, thin, elastic and capable of being stretched extensively with needles without tearing, while the lungs from infected frogs are less spongy, more brittle and more readily torn. Although the two conditions have been described as being very distinct there is in reality a gradation of the one into the other. The thickening is apparently caused by the activities of these parasites which literally bury their anterior ends in the reticulum of lung tissue and feed on blood. Since their bodies are covered with spines their movements must cause many abrasions of the lung tissues, however, infected lung tissue has not been studied histologically. Von Linstow (1890) found that occasionally in heavy infections with *Haplometra cylindracea* there was a chronic inflammation.

Incompatibility apparently exists among lung parasites. Roundworms of the genus *Rhabdonema* are common in the lungs of the frogs in the region studied. These worms are sometimes found living with the flukes in the lungs, but usually if the roundworms are numerous the flukes are few or absent altogether, or the opposite condition prevails. In several instances of heavy infections with both nematodes and trematodes in a single host, the roundworms were confined to one lung and the flat worms to the other. In some of my early work frogs experimentally infected with lung flukes were kept individually in fruit jars with very unsatisfactory results. Nematodes were invariably present in large numbers and trematodes almost always absent, which seemingly indicated favorable conditions for the growth of roundworms, but unfavorable for the flukes.

Experiments with Eggs and Miracidia. *Pneumonoeces medioplexus* has an extensive uterus filled with vast numbers of eggs, which when discharged from the fluke contain mature miracidia. After discharge, the eggs are carried in the fluids of the lungs by ciliary activity through the glottis into the mouth cavity. Then they are swallowed, passed through the digestive system and voided with the feces. In this way many of the eggs are deposited in the shallow water along the margins of ponds, lakes and other bodies of water where snails are usually abundant. The miracidia within the eggs have been kept alive for two months in water in a watch-glass. Some eggs that were kept for several months in small vials showed dead shrunken miracidia. Most of the eggs are so brown that it is difficult to see the living miracidium in them. Miracidia can be seen only in the few eggs whose shells are very light in color. Numerous eggs have been examined under the oil immersion lens for evidence of life in the enclosed miracidium and in a few instances occasional contractions of the body were observed. In eggs that pass unopened through the digestive system of a snail the movements of the miracidium are frequent and the cilia beat. Although the cilia are long in proportion to the size of the miracidium they are very difficult to see through the egg membrane.

Eggs are recovered from the parasites by putting them in water which

should be cold, if the specimen is to be preserved, for the cuticle is not so apt to slough off. Several minutes after the initial activity the parasites become motionless, contract, and relax in 10 to 30 minutes, whereupon the eggs begin to escape from the genital opening in a continuous mass held together by a secretion. They are discharged at a nearly uniform rate sometimes for an hour or more but the last eggs (young eggs) do not contain fully-developed miracidia. A few minutes after the eggs have escaped the mass falls apart and great numbers of them can be seen as brown patches when the dish is placed on a white background. After the parasite is removed, if the eggs are to be fed to snails, it is well to distribute the eggs over the bottom of the dish by shaking it or by using a dropper. If the eggs stand for two or three days in a covered water-glass they adhere to the bottom of the container.

The eggs do not hatch in the open as do those of many well-known trematodes. Various methods to induce hatching of the eggs, such as pressure on the cover-glass, change of temperature, freezing and subjection to artificial digestive juices have been tried without success. To cause the eggs to hatch they must be fed to snails. *Gyraulus parvus*, young *Helisoma trivolvis*, and *Planorbula armigera*, the host snail, have been used for this purpose and many of the eggs hatched while passing through the digestive system. Since an infection has never been found in the first two snails mentioned, it appears that the digestive action of any snail may stimulate hatching. *G. parvus*, when put in the dish with the eggs, usually scrape them up in large numbers and devour them at once. *H. trivolvis* and *P. armigera*, however, are very cautious and exceedingly sensitive to vibration and handling; therefore, much care must be used in coaxing them to eat. If newly-voided parasite eggs are used in the feeding experiments the currents caused by the snail's cilia literally "blow" the eggs away in all directions. The pigmentation of the adult snail is usually too great to permit observation of what happens to the eggs in their passage through the intestine, but in *G. parvus* and *P. armigera* a steady stream of them can usually be seen passing down the esophagus aided by action of the cilia. In 15 or 20 minutes the snail voids feces consisting of nothing but eggs surrounded apparently by a mucous covering. When the feces are deposited most of the miracidia have escaped from the open eggs. If a bit of the excrement, immediately after being void, is mounted on a slide and examined, miracidia will be seen "worming" their way out of the cylinder of excrement, but many never escape. When very young snails are fed upon eggs the miracidia do not have difficulty in freeing themselves because in these molluscs the mucous layer is very thin. In the miracidia that escape as well as in those imprisoned by the mucus the ciliary action becomes progressively slower, while they continue to elongate and contract until death. The few remaining in the opened eggs can be seen attempting to escape by the combined action of cilia and worm-like contractions and elongations of the body. No miracidium has

ever been seen actually making its escape from an opened egg in the feces of old snails but this was rather commonly observed in feces of young snails. Whether this is merely a coincidence or there is a definite difference in the snail or its feces that accounts for this was not certain.

In the opened eggs the operculum frequently is entirely removed. In some instances it is wide open and only fastened by a mere fragment, and in others the margins in contact are rather long and the operculum only slightly opened. The suture line is undulating.

In order to secure more information about hatching, the eggs were fed to 3 to 5-day-old laboratory-raised *Gyraulus parvus* and these were observed under the microscope. These small snails had a shell measurement of 0.436 by 0.350 mm. They were more wary than the half-grown ones and the adults. Usually if disturbed several times they would not eat but would try to escape. If fungous or bacterial growths were too abundant around the eggs the snails avoided them and sought places where the layer was thinner and the eggs fewer. As a rule the newly hatched snails of this species were quite transparent except for the region around the stomach which contains a quantity of black pigment. The eggs could be seen going through all except the stomach region of the digestive system and could be counted as they passed through the esophagus, but when they reached the stomach they became crowded together. If there were many eggs they were retarded at the stomach and passed through at a fairly regular rate. Consequently they were moved back and forth in this region by the muscular contractions of the esophageal wall and by the action of the cilia which lines the esophagus as well as the intestinal tract. The stomach also underwent rhythmical contractions and apparently only a few of the eggs at a time were subjected to pressure by these contractions and finally passed on to the intestine. Since neither miracidia nor opened eggs have ever been observed anterior to the intestine it is thought that the treatment received in the stomach had something to do with the hatching of the eggs. In the intestine, as in the other parts of the digestive tract, the eggs and the escaped miracidia were moved back and forth by the ciliary and peristaltic actions. The cilia on the freed miracidia in the intestine beat energetically but did not seem to aid the larvae noticeably. If the small snails continued to eat eggs rather steadily, the first eggs and freed miracidia were voided in about 12 minutes. The fecal mass was literally shot out of the anus with great force which was created apparently by the action of cilia at the terminal end of the digestive tract. After the escape from the snail the cilia on the miracidium continued to beat and the body of the larva underwent contortions. The miracidia made little or no progress in the water and most of them attached themselves to the dish by the mouth region which is adhesive. The surface layer of the miracidium soon became vacuolated, the action of the cilia slower and soon the larva was dead. Many times before attaching themselves to the

dish they were caught in the currents of water produced by the snail's ciliated body and were thrown against it but the miracidia did not respond to these stimuli.

There was very little evidence to indicate that in this and in closely related species of lung fluke the miracidia penetrate into the snail after being voided in the feces. Evidence is given for this conclusion in more detail in the part dealing with the experimental work on miracidia of *P. parviplextus*.

In feeding parasite eggs, half-grown snails are the most desirable since they do not notice handling and usually begin eating as soon as they are placed in a dish with eggs.

Sporocysts. The sporocysts which produce the cercariae live in the liver and were studied by dissecting infected snails. It is assumed, until further evidence is obtained, that these are daughter (second generation) sporocysts. The only experimental work done in this connection was to infect snails as described in the part dealing with the final experiment for this species.

Experiments with Cercariae. To obtain cercariae for study and infection purposes snails, *P. armigera*, were collected from regions in which adult flukes, *Pneumonoeces medioplextus*, were known to occur in abundance in leopard frogs. Such a region was found in a swampy area called Fontinalis Run on Burt Lake, near the University of Michigan Biological Station.

For the determination of infected snails they were placed individually in water in watch-glasses, left from 12 to 24 hours after which the water was examined under a wide-field binocular dissecting microscope. The wide-field instrument was required to find the few small cercariae which escape from the snail. Snails from which cercariae escaped are referred to as active infections in this paper. During the summer of 1929, accurate data were kept on the collections made and the following results were obtained.

In the manner outlined above, several hundred snails were collected on June 29, 1929 and 140 from this group were examined. Of these 34 (24 per cent) were infected. The remainder of the snails was placed, the same day, in a small rectangular aquarium and two days later trematode-free *Sympetrum* nymphs were added. On August 5, when cercariae were very badly needed all the snails in the aquarium, 650, were examined, but no active infections were found, although metacercariae were numerous in the nymphs, showing that some of the snails had been shedding cercariae. At various intervals during the summer additional collections of snails were made from Fontinalis Run and examined for active infections. On July 19, forty snails were taken and two had infections. On July 29, one active infection was found in 99 snails. No infections were obtained from 45 snails on August 3. The swamps from which all of the snails were taken, slowly dried up and by fall there were very few snails as compared with the numbers gathered early in the summer. This drying up probably was responsible for the decrease in the percentage in-

fects. The snails with active infections in nature as well as those in the laboratory decreased with the advance of the season as these data indicate. No double infections were found in this species of snail.

The infected snails were kept individually in shell vials (capacity 40 cc.). The water and food (decaying lettuce) were changed daily. Cercariae were never given off in large numbers, but in the heaviest infections approximately 300 escaped daily from the snail. This continued for one or two days, after which no more than 50 cercariae were eliminated.

In snails giving off so many cercariae the infection was far advanced and the liver was almost entirely destroyed. Brown (1926) says that snails infected with echinostome or stylet cercariae die sooner than those infected with fork-tailed ones. Two snails with lung fluke infections shed several cercariae daily for a month but these were exceptional cases. Snails usually discontinued shedding after two weeks which caused difficulty with experiments. A few cercariae escaped from the snails throughout the day but most of them were discharged during the night, which was true also of cercariae shed by experimentally-infected snails. In the cool of the morning the cercariae swam sluggishly near the bottom of the container, therefore in order to concentrate them for use nearly all of the water could be poured out of the vial without losing a specimen. When the water temperature rose above 75°F., the cercariae became active and well-distributed. There is no noticeable heliotropic response in these cercariae. They distribute themselves uniformly through the water and are not influenced by decreasing or increasing the light in some part of the container. Most of the time they swim aimlessly about lashing their tails but make no definite progress. Swimming keeps them suspended in the water, a function which is of great importance in bringing them into association with dragonfly nymphs, the next intermediate host. At times the cercariae creep by means of their suckers, in measuring-worm fashion. When creeping the tail is contracted, except for a periodic elongation during which there is a short period of lashing. This is especially noticeable when the cercariae are placed in a drop of water under a cover-glass. When handled in this manner they creep very rapidly by means of their suckers and progress by turning themselves over and over, attaching the suckers alternately to slide and cover-glass. As the water evaporates the movement is retarded, at which time there is a short period for studying the cercaria before it falls to pieces.

The second intermediate host, the dragonfly of the genus *Symptetrum*, receives its infection during the larval period. Dragonfly nymphs respire by means of a unique respiratory organ, called a branchial basket, which develops from a portion of the rectum. This organ is a large barrel-like chamber from the wall of which longitudinal sets of leaf-like gills or lamellae project inwardly. Since the entire organ is lined with chitinous cuticle, each lamella is covered by a thin layer. Respiratory movements cause water to pass in and out of the

branchial basket through the anal opening. These powerful movements, which are usually more or less rhythmical, create currents of water around the nymph, the direction of which the larva is capable of changing.

Cercariae swimming in the vicinity of the nymph are caught by these respiratory currents and are swept into the chamber of the branchial basket through the anal opening. When once they enter, it is very rare that the cercariae reappear, except when the nymph is not *Sympetrum*. Even in this case a short period of time elapses before they make their reappearance by being swept out of the opening. Doubtless, cercariae are thus swept into the branchial baskets of many kinds of dragonfly nymphs, while many cercariae never enter a nymph. The latter cercariae usually die within 30 hours after they escape from the snail and those which enter nymphs other than genus *Sympetrum* also die. Those which are taken into the respiratory organ of *Sympetrum* nymphs penetrate through the chitin, into the tissue of the lamellae or wall of the respiratory organ, and occasionally go all the way through it and encyst.

A single cercaria has been seen which had penetrated entirely through the wall and encysted. This was in a very small nymph infected in the laboratory. From its position in the nymph and the looseness of attachment of the cyst one might conclude that it is possible for an occasional cercaria to stray to some other part of the body. In nymphs the cysts, however, have never been found any distance from the branchial basket, and in the insect after transformation they have always been found loosely attached to the vestige of the respiratory organ in the posterior end of the abdomen. If cercariae stray away from the branchial basket after penetration the condition is very unusual, judging from the number of specimens which have been dissected and the result of the following experiment.

Four frogs that had been collected from nature were kept under controlled conditions for eight weeks which permitted any lung flukes already in the frogs to mature. Then *Sympetrum* adults were collected in which there was nearly 100 per cent infection. The posterior end of the abdomen, where the cysts have always been found, was clipped off and the remainder of the insect was fed to these frogs. During a period of four days, several hundred freshly-operated insects were forced into the mouth of each frog. At the end of this time when they were examined, the frogs were negative for flukes, a finding which supported observations on the position of the cysts in the body of the nymph and adult dragonfly.

Various experiments were conducted with cercariae and dragonfly nymphs. The *Sympetrum* nymphs for infections were obtained from two sources. For the early experiments they were taken from an acid lake in which there were no closely related dragonfly genera. This acid lake was free from snails as

shown by observations of various investigators during the last few years. Jewell and Brown (1924, 1929) and Brown and Jewell (1926) have reported limnological studies for this lake from 1923-1926 without evidence of snails. Professor P. S. Welch, University of Michigan, has informed me that he and his students have made extensive observations on this lake for six years with the same result. I made numerous collections of nymphs along the shore of this lake with dredge nets during the summers of 1928 and 1929 without finding evidence of snails. The absence of snails from the lake permits the assumption that nymphs as well as the tadpoles living in the lake should be free from trematodes. All examinations of such specimens verified this conclusion. Extensive collections of nymphs and tadpoles were made from this lake during the summers of 1928 and 1929. When these specimens transformed they were examined for trematodes and all were negative. The adult insects and small frogs were also collected from the lake during this time and they, too, were negative. The latter condition, it must be admitted, was rather unusual but one familiar with the lake would, no doubt, conclude that the lack of infection in these hosts was due to isolation from bodies of water in which snails lived. Even though it seemed needless to run controls for experiments, in which nymphs from this locality were used, it was done.

Other *Sympetrum* nymphs employed in the experiments were raised in the laboratory from eggs according to methods published (Krull, 1929). These were used for final as well as other experiments, and controls were maintained for them. Better results were secured with these laboratory-raised nymphs as the metacercariae were more uniform in size. In some experiments the nymphs were infected by placing them in 40 cc. shell vials containing the snail, and left for several hours; in others the nymph and snail were left together over night. In comparing the two methods a decided difference was found in the number of metacercariae in the nymphs; in the former they were much more numerous. This, no doubt, is a direct result of differences in temperature. Under what may be considered optimum conditions the percentage of cercariae which entered the insect and developed into metacercariae was large. The temperature of water and the age of cercariae influenced greatly the success of experimental results. Infection experiments conducted at 68° to 70°F. were not as successful as those at 75° to 80°F. The most accurate and successful method of infecting nymphs consisted in using a very small container made by cutting off the bottom part of a two-dram vial to a depth of about one-half inch. By means of a wide-field binocular microscope cercariae were counted as they were taken from a watch glass by a small-mouthed dropper, and transferred to the receptacle. As many as 250 cercariae have been placed in the vial with a single nymph and invariably when the water was warm one-half hour was sufficient time for all of these larval flukes to be taken into the nymph.

Sometimes it was necessary to infect nymphs on cool evenings, or on cold, rainy days and, in spite of the close proximity of the insect and cercariae, it was impossible without great effort to get any of them into the nymph. The swimming of the cercariae was so sluggish that they did not rise in the water. Since the anal opening of the nymph was at an angle of about 45° with the bottom of the container the respiratory currents were not rightly directed to affect the lower layer of the medium in which the cercariae lay and thus infection was prevented. When such a condition prevailed the nymphs were turned on their backs and held in this position with a pair of forceps. This was one method of forcing the cercariae into the nymph, but later when the dragonfly larvae were examined, few metacercariae were present. Thereafter when nymphs had to be infected under conditions of low temperature the vial containing them and cercariae was placed in a warm water bath which increased the activity of the cercariae almost immediately. They soon became distributed vertically as well as horizontally in the water. Good infections were secured by this method. On several occasions while the nymph was being infected the water became too warm, whereupon the nymph relaxed and ceased to respire, and the cercariae discontinued activity and dropped to the bottom. In all cases that were observed the nymphs recovered when the water was cooled and only a small percentage of the cercariae died.

Whether the thickness of the chitin lining the branchial basket has any effect on the penetration of the cercariae has not been definitely determined on account of the dearth of nymphs and larval flukes at the proper time. The evidence at hand, however, indicates that thickness of the chitin does not affect their entrance. As proof several nymphs, three days before their transformation, were exposed to cercariae, and when they were examined, heavy infections were found. If one assumes that the nymph at this time has, perhaps, the thickest layer of chitin of its larval period it must be admitted that this covering has little or no effect on penetration.

The factors discussed in the foregoing paragraphs do not explain all the irregularities of infections in the nymphs. In the experiments 75 nymphs have been infected individually in determining this life history. Observations showed that there are rather decided differences, perhaps physiological, in the nymphs of the same size in the same species. When one finds that of two nymphs of equal size hatched simultaneously from the identical batch of eggs and both infected at the same time with cercariae from one snail, the one nymph having many large well-formed metacercariae, and the other but few small and poorly-formed metacercariae, he is forced to conclude that internal conditions were the cause. Experimental evidence proves that the size of the nymph does not affect its susceptibility, it only conditions the space for cercariae and the number of them that it can endure at any given time. Nymphs cannot be infected during the first instar, pronymphal stage, because the entire nymph is sur-

rounded by a chitinous pronymphal sheath and the respiratory organ is not functional during this time. This period, however, only lasts a few minutes so one may say that nymphs can be infected from the beginning of the second instar up to the time they leave the water, an infective period of over two months as shown by nymphs raised in the laboratory (Krull, 1929). Natural infection of nymphs in the second instar, as determined by their size, was discovered on April 30, 1929, when trash was collected from a small pool near Ann Arbor. In examining this material three *Sympetrum* nymphs were recovered and isolated in fruit jars and kept in the laboratory away from possible sources of infection. They transformed in July at which time they were examined for encysted metacercariae. Two of the dragonflies were negative, but the third contained five large metacercariae. The laboratory infection experiments with nymphs in the second instar are described in the studies on *Pneumobites parviflexus*.

Nymphs of any size can be killed by allowing them to become too heavily infected. Three cercariae are sufficient to kill a nymph in the second instar, if they are all taken in at about the same time; for large nymphs hundreds are required. No nymph that lived through a heavy infection was exposed to more than 250 cercariae and in several cases this number entered in one-half hour which shows that the nymph can withstand a large number. The nymph, having received an infection greater than it can sustain seems to be more or less paralyzed, unable to move or eat and dies within a day. Medium-sized nymphs as a rule are more affected by the active entrance of the cercariae into the tissues, than the large ones, although a great deal of individual variation exists.

The activity of the nymph during the infection process is both interesting and amusing to observe. A typical observation record follows. As soon as the nymph was set free in the small container and noticed the presence of so many moving creatures, it seemed to be frightened. It remained motionless, stopped respiring for a period of seconds and watched the strange wiggling creatures. Finally it caught and tried to eat the cercariae but was unable to hold them as they squirmed out through the mouth parts and swam away. Soon the nymphal respiratory movements began and the cercariae were sucked into the branchial basket. The dragonfly larva immediately sensed these as foreign particles and tried to expel them by a very vigorous exhalation, as when any unusually large foreign particle entered. After the unsuccessful attempt the anal opening was promptly closed and the branchial basket could be seen going through powerful, convulsive contractions. Finally due to necessity, no doubt, respiration was restored but usually with a different rhythm or depth of movement. Cercariae were also crawling on the body and appendages of the nymph which the insect very energetically tried to rub off with its legs and was sometimes successful. Added cercariae in the respiratory chamber had by this time caused the nymph to again dispense with respira-

tory activity for a few seconds.* In five minutes the nymph showed other symptoms. The pigment in the eyes changed its position and was contracted which affected the expression of the face and gave the insect a "wild" appearance. The intestine was full and was evacuated with great rapidity. Then by the aid of the respiratory organ for propulsion the nymph literally sailed around the dish, stopped for a second and then began to run and tried to break through the side and bottom of the container but curiously enough in this case as in all others, it did not leave the water and go over the top. After such activity for a few seconds it flipped itself over on its back and tried to use its legs, especially the posterior pair, in scratching its abdomen. During this time respiratory movements were again suspended for a brief interval. Then the labium was thrust out a number of times in rapid succession. In some extreme cases the nymphs completely relaxed for several seconds. Whether these movements were symptoms of fatigue, paralysis, pain, or toxins** has not been determined. In five minutes the nymph righted itself and its activities became more and more normal even though other cercariae were entering. This fact which was observed repeatedly while infecting nymphs indicates to me that toxins are probably not involved in producing the behavior, pain and fright as causes seem to me a more plausible explanation.

The behavior of the other nymphs in infection experiments of a like nature was very much the same but a few differences should be mentioned. Once in a while the nymph raced around in the dish by using the respiratory organ, then stopped and changed the rate of respiration, and by occasional powerful contractions tried to expel the foreign objects. In several instances, the nymphs elevated the tip of the abdomen and squirted several drops of water as far as seven inches which gives an idea of how powerful this organ really is in these comparatively delicate larvae. Occasionally as they recovered from being infected they violently shook the abdomen from side to side as do small ducks when emerging from water. A reflex, a sort a jerking movement, more or less periodic was usually set up in the hind legs, and had the appearance of being an attempt to scratch the abdomen. These two activities sometimes continued for a half hour or more. If the nymphs were hungry and food was present they ate almost any time after the initial performance.

The pronounced activity of the nymph during the infection is decidedly effective in drawing the cercariae into the host since it is a passive entrance

* Since there are tubercles on the lamellae which hold them apart it seems that the cessation of respiratory movements gives these cercariae a good opportunity to crawl up between the lamellae to, or at least near, the wall of the organ where they usually penetrate. The tubercles also probably prevent the nymph from killing the cercariae by pressure.

** Ssinitzin (1907) observed such conditions in dragonfly nymphs infected with frog bladder flukes when cercariae were penetrating the gut of the nymph which he claimed was due to paralysis as a result of toxins but it seems that this would be rather difficult to determine.

on their part as the host really infects itself. Nymphs aid in obtaining an infection by establishing and directing currents of water and by changing the position of the guides in the anal opening. They do not, however, always use this mechanism.

In nature the dragonfly nymphs may become infected very early in the spring; they transform and retain the metacercariae throughout the summer. In the foregoing pages a case was cited in which a very young nymph became infected before the first of May. *Sympetrum obtrusum* which begins to fly the latter part of June carries fully developed metacercariae. If collections are made from time to time during the flying season in a favorable locality it is found that the percentage of infection as well as the number of metacercariae in the individual increases. Metacercariae live in the insect until it dies in the fall, unless it is eaten by the final host. Living metacercariae have been recovered from adult dragonflies as late as October 18.

The specificity of the cercariae to the dragonfly host is very pronounced. Metacercariae have never been found in any thing but dragonflies of the genus *Sympetrum*. Specimens of *Leucorrhinia*,* a genus closely related to *Sympetrum*, have never been found to carry metacercariae of frog lung flukes; although the nymphs of some of the species in these two genera are externally, at least, morphologically indistinguishable. In carefully conducted infection experiments an attempt was made to infect nine nymphs, genus *Leucorrhinia*, without success. In one case a nymph from each genus, *Sympetrum* and *Leucorrhinia*, was exposed to cercariae at the same time in the same container and afterwards examined on the same day; *Sympetrum* was positive for metacercariae while *Leucorrhinia* was negative.

Experiments with Metacercariae. Dragonflies may carry large numbers of metacercariae. Since the cysts are found in a very limited area of the abdomen of the teneral or adult, such an infection actually produces a bulging of the abdominal wall of this region. The largest number of metacercariae which has been counted in any dragonfly was 220. This was a teneral of *S. obtrusum* collected July 30, 1929.

Comparison of *Sympetrum* which were taken from many localities in the Douglas Lake area and in the Ann Arbor region shows the infestation to be much greater, as to numbers in the individual and percentage of individuals infected, in the former region. At Fontinalis Run on Burt Lake, there is in the month of August in a favorable season almost a 100 per cent infection in *Sympetrum*. During the first two weeks in August, 1928, 64 specimens were collected from this place and 61 were positive. Almost every one of those infected carried more than 100 flukes apiece. A peculiar instance of dragonfly infestation was found on the shore of Douglas Lake. A pool was discovered

*This was probably *L. proxima* Calvert wherever referred to in this paper since those which were kept until transformation were identified as this species.

near the edge of the lake which was inhabited by the desired species of frog and snail. The adult *Sympetrum* flying round this pool were exceedingly numerous, but the proportion of those infected was decidedly small when compared to the large percentage of infection in nymphs in the pool. This immediately became a subject for inquiry and a much larger pond, ideal for dragonfly nymphs, was discovered in a densely-wooded area, located 50 feet from the other pond. Nymphs examined from this pool were all negative. As there were only a few snails of any kind in the pool, this discovery accounted for the irregularities of infection which had been observed.

In the dragonfly nymphs, metacercariae ordinarily attain maximum size in 14 to 20 days and when their growth is completed they are 4 or 5 times the size of the body of the cercariae. They are infective, however, when much smaller; are known to be infective after a 6 days' sojourn in the nymph, and may be infective even earlier. Some metacercariae apparently never attain maximum size. These are invariably found in the lamellae in flattened cysts and probably the stunting of the growth results from mechanical difficulties encountered in that structure.

The metacercariae are found in thin, transparent, and pliable cysts, and as stated they are located in various parts of the branchial basket. In the nymphs collected in nature the majority of the cysts have been found in the lamellae. In heavy infections there are as many as six cysts in a single lamella. In laboratory infected nymphs the majority of cysts are in the wall of the branchial basket. The encysted larvae remain in the thin-walled cysts in this position until transformation of the nymph. Distortion of the lamellae and the entire branchial basket is brought about by the increase in size of the metacercariae. During transformation the nymphal respiratory organ breaks down. It is absorbed except for a short region which remains as a collar-like thickening in the posterior end of the digestive tract of the adult. When metacercariae are present they are crowded together and attached to this vestige of the nymphal respiratory organ. In the transformation process the cysts along with the host tissue are withdrawn from the chitinous envelopes of the lamellae. These coverings are discarded with the exuvia. If a teneral is dissected immediately after transformation one finds a jelly-like mass containing pigment which marks the position of the nymphal respiratory organ. At this time the cysts are more or less buried in this pigmented mass. As the insect grows older this vestige becomes smaller and the cysts become more and more exposed until in the old mature specimens all the pigment, originally in the lamellae, is usually gone and the vestige, except for the cysts present, is not easily seen.

Since the nymphal respiratory organ is so complicated, containing hundreds of individual lamellae in large nymphs, the method of examination may be of interest. In some cases the nymphs were kept until transformation and then

the autopsy was easily made. With a pair of scissors one clips from the abdomen of the dragonfly the three posterior segments, which contain the cysts, and with teasing needles separates the terga from the sterna in this piece to expose the infection. In freeing the cysts from the nymphs a pair of fine, sharp scissors was used to cut a narrow longitudinal strip from each side of the abdomen. Then the posterior end was cut off and the abdomen, severed from the rest of the insect, was dropped into normal saline solution. Under a wide-field binocular microscope with a pair of fine teasing needles, the terga can be easily separated from the sterna in one piece and the branchial basket lifted out. The cysts can now be freed with a pair of teasing needles by pulling the basket apart and teasing the cysts out of the lamellae. The larger cysts in water or saline will usually open in a minute or two without any additional aid, but ordinarily the small ones remain intact and have to be opened. The metacercariae are so delicate that it is almost impossible to open the smaller cysts without injuring the parasites. With the following method excellent results were secured. When the basket is freed from the nymph it is dropped into artificial gastric juice. In 45 seconds all of the metacercariae will be freed from the cysts, and can be transferred to saline solution. Cysts from adult dragonflies can be treated in the same way. Metacercariae will live in saline solution under conditions where it is not too warm for as many as 12 hours.

The metacercariae in the living insect are always encysted. If insects are opened several hours after death some metacercariae are found which have freed themselves from the cyst. If ordinary dissecting methods are used in freeing these larvae from hosts some of the cysts are usually opened, but by careful dissection of a series of adult insects it was concluded that normally all of the larvae are encysted in living specimens.

When cysts are first exposed in a nymph or adult the metacercariae are inactive but when the encysted metacercariae are dropped into saline solution or water they become active and this, no doubt, helps open the cyst. When put into artificial gastric juice there is a very definite response; the metacercariae immediately become exceedingly active, and during this activity rapidly elongate and contract. Water gradually slows down the activity in about an hour. If metacercariae are on the bottom of a container or on a slide under a cover-glass they make little or no progress because they do not use the suckers as do the cercariae. Only in rare cases have they been seen using these organs under such conditions.

Final Experiment for Life History Determination. Practically nothing was known of the life history of the frog lung flukes belonging in the genera *Pneumonoeces* and *Pneumobites* when this work was started. The cercaria and the snail host are not mentioned in the literature for any of the species in the two genera. Ssinitzin (1907) according to Cort (1915a) found

stages of *Pneumonocces variegatus* free in the body cavity of both nymphs and adults of the damsel-fly, *Calopteryx virgo*. By feeding these insects to frogs he was able to infect them, showing that the damsel-fly was the second intermediate host. Stafford (1902) mentioned immature flukes in one of his species which were only one-half millimeter in length. Cort (1915a) taking from frogs' lungs immature stages, gave an adequate description of young *Pneumonocces coloradensis* Cort, the smallest of them having metacercarial proportions.

The life history of *Haplometra cylindracea* (Zeder) closely related to *Pneumonocces* and *Pneumobites*, apparently not found in North America, was described by Von Linstow in 1890. The brief description of the life history on the basis of comparative morphology as in most life history descriptions of the old order is given with no mention of feeding experiments. This was the extent of the knowledge concerning the life history of frog lung flukes when this problem was begun. The life cycle of *medioplexus* raised in experimental hosts follows.

Since it was necessary in the preliminary experiments to use some parasite-free hosts taken in nature it was thought best, in order to give a critical analysis of what had been accomplished, to perform a final experiment in which snail, dragonfly, and frog hosts were laboratory-raised from eggs. This is called the final experiment and is here described. This experiment was begun on June 29, 1929, and was completed September 20, 1930, a period of 448 days. It started with the collecting of an infected *Planorbula armigera* from the swamp on Burt Lake near the Biological Station. This snail was one of two which discharged cercariae for a comparatively long time. From this snail cercariae escaped for 25 days, some of which were preserved in 5 per cent formalin. Others were used to infect a nymph of *Sympetrum*, about two-thirds grown, which had been collected July 14, 1929, from an acid lake. This nymph was exposed two days later by leaving it over night with the snail which was actively shedding cercariae. It was examined 21 days later and 65 metacercariae were recovered, some of which were preserved while others were fed to frogs. The head and thorax of the nymph were preserved for identification.

On August 6, 1929, twelve of the metacercariae in tissue were fed to a small leopard frog which was then isolated in a covered pen. This frog had been collected as a tadpole July 22, 1929, from an acid bog and had transformed in an aquarium. All of the above was done at the Biological Station; therefore, when the Station closed, the frog was taken to Ann Arbor where it was placed in a covered aquarium and fed on earthworms during the fall, winter, and spring. At the time of examination, 285 days later, the frog was 42 mm. long. Three adult *P. medioplexus* were recovered from the lungs. These were placed in a watch-glass where the eggs were emitted. In an hour the para-

sites were removed, fixed and preserved. The dish containing the eggs was covered and put aside for several days in order that they might adhere to the bottom so that they would not be shifted by the cilia of a snail when the latter was introduced.

The eggs were fed to 15-day old *P. armigera* that had been raised in the laboratory. The progenitors of these snails had been collected in March from a pool near Ann Arbor. An attempt was made to feed each of the 15-day-old snails a few eggs while it was being watched under a microscope. This method had to be abandoned after several hours because the snails had been used without success; they were too wary and sensitive to vibration to be so handled. While some snails remained active in spite of being disturbed, only two ate eggs. Therefore, the entire number was placed in the dish with the parasite eggs and left for several hours. At the end of this time a large amount of feces consisting solely of eggs was evidence that some of the snails had been eating. The snails were then transferred to an evaporating dish with tap water and dead maple leaves. They grew well but when compared with two dishes of control snails it was discovered that their mortality was exceptionally high during June and July. The latter part of July those remaining were examined several times for active infections but all were negative. On August 2, 1930, the five remaining living snails, half grown, were examined for active infections. On the morning of August 3, 1930, three cercariae were found in the dish, but no more were shed during the day. One cercaria escaped on each of the following mornings, August 4 and 5, but no more emerged throughout these two days. Since it was necessary to be absent from Ann Arbor for a time it was thought best to crush and examine the snails for infections. Three of the five were infected, one was an active infection and the other two were nearly mature. Forty-two control snails, laboratory raised, were kept in another dish and when examined were free from infections. The escape of cercariae from the host during the night seems to be a constant characteristic for *P. medioplexus* since it was observed in experimental as well as natural infections. Just how much time is required for cercariae to mature cannot be determined from this experiment. The snails invariably carry some eggs on the shell and since the miracidia remain viable for weeks there is a long period of time during which the eggs may be eaten. However, since they ate so freely this carrying of eggs on the shell may be disregarded. The experiment proves that no more than 65 days is needed for production of cercariae.

The three cercariae recovered on August 3, 1930, were placed in a small container of water, and a young nymph, 3 mm. in length was introduced. The infection experiment was watched until the three cercariae were swept into the branchial basket by currents created in respiring. Characteristic responses to the penetration of the cercariae were observed in the nymph. An-

other nymph of the same size was used for the cercaria shed on August 4 and 5. At each exposure the nymph was watched until the cercaria disappeared. This nymph and the one mentioned before were *S. obtrusum*. The eggs from which they hatched on July 26, 1930, had been collected in August, 1929. Such eggs normally hatch in April or May but deferred hatching was accomplished by keeping the eggs frozen in ice in a refrigerator. These nymphs were fed on laboratory-raised *Paramecium*, *Cyclops*, and *Tubifex*. After infection, the living nymphs were examined for developing cysts and one was observed on the dorsal side of the branchial basket of the nymph which had been exposed to the three cercariae.

On August 14, 1930, when the metacercariae were nine to eleven days old, the nymphs were fed to a small laboratory-raised leopard frog, which was isolated in a fruit jar in a small amount of water for several hours before returning it to the aquarium room, to make sure that the nymphs had been retained. The frog was the sole survivor of three which had metamorphosed in the laboratory from tadpoles raised from eggs collected in the spring of 1929. The tadpoles were kept in cement tanks in the laboratory and fed on lettuce. Immediately after transformation the frog which lived was small, emaciated, and so weak that it could not feed itself. It was fed small pieces of earthworms every other day for more than a month. Thereafter it was able to catch fruit flies which were breeding in the aquarium room in decaying lettuce and were attracted to the frog's aquarium by pieces of apple.

This frog, only 31 mm. long, was examined on September 20, 1930, thirty-seven days after it had eaten the infected nymphs. One small mature *P. medioplexus* was recovered from the lungs. The frog and fluke were preserved.

In this experiment the parasite was carried uninterruptedly under laboratory conditions, through one complete cycle, involving snail, dragonfly, and frog, all of which were raised in the laboratory from eggs and fed on laboratory-raised or trematode-free food at every stage of development. This experiment, with those related under the topic, Biology of *P. medioplexus*, establishes conclusively the life history of *P. medioplexus*.

BIOLOGY OF PNEUMOBITES PARVIPLEXUS (IRWIN)

Pneumobites parviplexus (Irwin) is a parasite found in the lungs of the green frog, *Rana clamitans* Latr., and has larval stages in the snail, *Gyraulus parvus* (Say) and certain dragonflies of the genus *Sympetrum*. *S. rubicundulum* (Say) and *S. obtrusum* (Hagen) have been infected in laboratory experiments. This parasite from the green frog has been found in no other species of frog. The fluke is quite common in Michigan in the regions which have been studied, and was first collected and recognized by me as a new species in the frogs of the Douglas region in 1928. Green frogs are not abundant in this

region and no specimen of *P. similiplexus* has been found there, but it has been taken elsewhere in Michigan. Fortner, who collected extensively in this region in 1917 and 1919, reported as *Pneumonoeccis similiplexus* Stafford from the green frog what is now known as *Pneumobites parviplexus*. These two species are so similar that they could easily be confused, although the characters by which they can be distinguished are constant. They differ in the length of the longitudinal folds of the uterus outside of the intestinal ceca, in the lobing of ovary, and in the size ratio of the oral to the ventral sucker. Irwin in her description of *Pneumobites parviplexus* gives the size ratio of the oral to the ventral sucker as 4:1. A specimen of the type material loaned to me by Dr. W. A. Riley, University of Minnesota, did not show the ventral sucker so I was unable to make a comparison. However, measurements of several of my series involving large numbers of flukes show a 3:1 ratio.

During the summers of 1928 and 1929, 14 green frogs were collected and three (21 per cent) were positive for *P. parviplexus*. Twenty-seven frogs of this species from the Ann Arbor region were examined in 1929 and 12 were infected. Nine is the maximum number of adult flukes taken in nature from a single specimen, and 80 is the largest number when all sizes from those of metacercarial proportions to large adults were considered. The frog that had 80 parasites was 78 mm. long and was collected on the fourth of September.

Observations were made on tadpoles in nature and on those used in infection experiments to find out whether they ever became infected with lung flukes. Twenty-six large tadpoles ready to metamorphose, were caught in a small pond from which the metacercariae of *P. parviplexus* had been discovered in the dragonfly nymphs, but examination of the tadpoles for lung flukes showed them to be negative. To determine the presence of the larvae of *P. parviplexus* in the nymphs from the pond, they were fed to two green frogs free from lung flukes. These frogs were examined about a month after feeding and lung flukes were recovered. The frogs used in the above experiment were collected as tadpoles from Vincent Lake, 1929, and were kept through the winter at Ann Arbor. An attempt was also made to infect eight large green frog tadpoles, taken from a lake near Ann Arbor. The nymphs that were fed to the tadpoles had been raised from eggs in the laboratory, and had been infected with cercariae from naturally-infected snails. With a dropper each tadpole was given about a dozen encysted metacercariae in nymph tissue and then put into a small container of water for two hours to see whether the cysts were regurgitated. One small green frog, raised in the laboratory from a tadpole collected in nature, was used as a control and was given 12 cysts. The tadpoles and the frog were examined 20 days after they had been given cysts. Seven flukes were recovered from the frog, but the tadpoles, were negative. Four other tadpoles, for which there was no con-

trol, were each given cysts from laboratory-raised and infected nymphs. These tadpoles were also negative for lung fluke infections. Tadpoles are not naturally infected and are not capable of being infected; therefore frogs which have transformed from tadpoles kept in the laboratory can be safely used for infection experiments.

A number of factors were instrumental in reducing the amount of observational and experimental data on this form. Discovery of the essential parts of the life history of this species was made after about two years of work had been done on *Pneumonoeces medioplexus*. At least a part of the life history of the former parasite was known for three summers in advance of the latter. It was therefore possible to make use of much information gained during the study of the other life history. The snail host of *Pneumobites parviplexus* was not discovered until early in the spring of 1929, and all of the experiments except the final one were completed the same season. Therefore, by the time this life history was found, much information gained while doing the previous work could be put to advantage here which saved considerable time. A method of rearing dragonfly nymphs had been completed and fortunately a sufficient number of nymphs was available for infection experiments when the snail host of this species of fluke was discovered. It was unfortunate that more trematode-free green frogs as final hosts were not available for use in the preliminary experiments. Frogs, except two, were collected and used from various localities as soon as they came out of hibernation in April. These were kept and used in the infection experiments and the difference in the size of flukes and the percentage of infection were criteria in determining whether the flukes found on examination were the result of natural or experimental infections.

Since the Biology of *Pneumonoeces medioplexus* is of a general nature such phases of the life history as hatching of eggs, method of infecting nymphs, method by which the frogs become infected, position of metacercariae in nymphs and adult dragonflies, method of escape of parasite eggs from the frog, migration of metacercariae in the final host and effect of parasite on the frog are the same in *Pneumobites parviplexus*.

In the infection experiments 27 green frogs were used and nine of these were less than 50 mm. in length. They were examined between 9 and 64 days after eating the metacercariae, and all were infected. This is much higher than has been found in nature. Fortunately one of these frogs had been collected as a tadpole from a lake on May 13, 1929. Two weeks later it metamorphosed and on June 6, was fed about 12 metacercariae from a nymph raised and infected in the laboratory. On the sixty-fourth day after feeding, the frog was examined and three adult flukes were recovered. This finding, together with the total infection obtained in the group of small frogs, proves conclusively

that at least some of the flukes found came from the experimental feedings. The largest number of adult flukes in experimental infections was recovered from a frog 71 mm. long which had been collected near Ann Arbor, May 15, fed from 100 metacercariae on June 8, examined on the fortieth day. There were 42 adult flukes but it was impossible to distinguish natural from laboratory raised parasites because all had attained maturity. Another green frog, 60 mm. long, was given about 100 metacercariae, and when examined 19 days later 61 immature flukes were found. There was a great variation in the sizes of these, some were nearing maturity, while others had made no growth.

Nymphs of *Sympetrum obtrusum* (Hagen) and *S. rubicundulum*, raised from eggs in the laboratory, were used in all the experimental infections. Sixteen were infected with cercariae secured from naturally-infected snails. Total infection resulted and in all cases cysts were well developed. This fact gives additional support for the statement made in the topic on, Biology of *P. medioplexus*, that the laboratory-raised nymphs were better for infection work than those collected in nature.

Dragonflies were collected from many Ann Arbor localities but they were not abundant enough in any region to be of significance for statistical work. The relative scarcity of cysts in dragonflies was correlated with the few infections which were found in frogs and snails in this region.

Snails were collected, handled, and infections isolated as in those used in experiments with *Pneumonoeces medioplexus* and most of them were taken from three localities in the Ann Arbor region. From an unnamed pool near Ann Arbor 900 snails were taken on April 8, and one active infection was found. On May 15, 81 were collected from the same pool and all were negative. On the same day 721 were collected from Weinsburg Pool, several miles southwest of Ann Arbor, and five infections were isolated. From Hall's Mill Race Pond near Ann Arbor, 216 snails were taken on October 12, and only one was infected.

In all my collections an average of less than one per cent of the snails had active infections. Several factors, no doubt, were responsible for this condition. All of these infections probably had been held over from the preceding year and it was too early in the spring for any new active infections. The three ponds, mentioned, were visited several times in the fall of the year and two of them had dried up after an unusually dry and hot summer. Whether due to drought or to other causes, the dragonfly in the fall was not flying in numbers. The limited number of infected frogs is quite likely another cause for the limited number of infections found in the snails.

While at the Biological Station in 1929, 101 specimens of *Gyraulius parvus* were collected in the swamp from which were taken all the *Planorbula armigera* that were used in preliminary life history experiments of *Pneu-*

monoeces medioplexus. The collection was made in July and three infections, three per cent, were found. Here the number of green frogs and snails was small but the dragonflies were abundant.

Final Experiment for Life History Determination. This experiment had its origin in six adult *Pneumobites parviplerus* taken from a green frog on March 8, 1930. After the parasites had been removed from the frog, they were put in a watch-glass of cold water where they remained until the eggs escaped. The parasites were then removed from the dish, fixed and preserved. The eggs were distributed in the dish by shaking, then it was covered, set aside and used for infection experiments with snails.

Some snails, *Gyraulus parvus*, were collected October 26, 1929, and kept in the laboratory all winter. They were isolated several times to determine whether they were infected, but no cercariae were ever found. In February they were placed in small shell vials where they laid their eggs during this and the following month. After eggs had been laid the snails were removed and the eggs were allowed to hatch. These small snails were raised under conditions which prevented any possibility of infection (Krull, 1931). Fifty-five of these, $\frac{1}{2}$ to 2 mm. in diameter and 1 to 15 days old, were infected on March 25, 1930, with parasite eggs taken seventeen days earlier from the flukes of the green frog mentioned in the preceding paragraph.

In the process of infecting the snails each was handled individually. It was placed with a dropper into the dish of eggs and then quickly brought into focus under the compound microscope and watched while it was eating. When the snail had taken from 10 to 200 eggs it was removed to a watch-glass of clean water. Sometimes the snails ate the parasite eggs so rapidly that it was difficult to get them out before taking too many. This was particularly true when there was a solid layer of eggs in the dish. They move the radula from 80 to 120 times a minute taking an average of five at a time, when the eggs are numerous. After the snails had been kept together in a watch-glass of clean water for an hour they were transferred to a larger container of water which had been supplied with several dried leaves as food.

On April 26, 1930, 31 days after infection, ten dead snails were taken out of the container. The soft parts had disintegrated in all except one which was dissected and found to be full of sporocysts, the liver was entirely destroyed and a few cercariae which were mature swam away. These cercariae, as will be pointed out later in this section, were used for infecting dragonfly nymphs. After dissecting this snail, one of the smallest which had made practically no growth was examined. It harbored sporocysts somewhat smaller than in the other infection. It contained no fully-developed cercariae but they had developed tailbuds. Since the two snails examined were positive, the rest were isolated to see whether cercariae were being shed. After the remaining 21 snails had been isolated for 24 hours only a few cercariae were

found. After this, death reduced the number of snails very rapidly. Only eight were left by May 12, 1930, when again they were isolated for 12 hours, and 300 cercariae were shed. One snail died while in the dish; the circulatory system had ruptured, filling all the lymph spaces with blood and the liver was entirely destroyed by numerous sporocysts. Three snails survived until August 3, 1930, at which time all were still producing cercariae in rather large numbers. One remained on September 8, 1930, from which only a few cercariae were escaping.

This part of the experiment brought out several interesting facts. It showed a high mortality among the snails, especially during the first month, at which time about two-thirds of them died. This number as compared with two containers of control snails was exceedingly high, and it is reasonable to conclude that the mortality was due to infections. It also showed that infective cercariae are produced in 32 days after snails have eaten eggs. Under natural conditions it is possible for the snail to become infected shortly after it hatches, to develop this infection while it grows to adult size, a period of a month or more, and then to produce cercariae throughout the summer.

In comparing the growth rate of *Gyraulus parvus* and the time necessary for the cercariae of *Pneumobites parviplexus* to develop in it, with the rate of growth of *Planorbula armigera* and the time necessary for cercariae of *Pneumonoeces medioplexus* to develop in it, it was found that both the rate of growth of the snail and the rate of development of the infection were more rapid in *Gyraulus parvus*. There is apparently a correlation in both of the forms between the rate of growth of the snail and time necessary for the infections to mature.

The infection of snails was repeated with another group. This time 41 thirty-day-old *Gyraulus parvus* raised in the laboratory, some of which were full grown, were used. They were exposed to infection April 30, 1930, and handled the same as those in the previous lot except that they were only allowed to eat from 5 to 15 eggs. It took longer to infect these snails because they had to be placed with only a few eggs and watched for a longer time. The parasite eggs used in this experiment were from the same batch as those used in the first one and had been kept in water for 53 days.

On May 19 and 27, 1930, the snails which had been exposed to infection April 30, 1930, were tested as usual for cercariae production by keeping them in a watch-glass for a 48-hour period and all were negative. The entire group of snails was not examined for cercariae production again until June 2, 1930, and at this time the cercariae were numerous. The remaining ones (30) were isolated individually June 19, 1930, and 22 of them had active infections. They were tested several times afterwards but this is the largest proportion of infection that was found at any time. The snails died one by one after June 26, 1930, and only a few were still living September 8, 1930, from which cer-

cariae were escaping. In this experiment, infections matured some time between 27 and 33 days and the peak of active infection apparently was reached in less than 50 days. During the first two weeks after the snails had been exposed to infection four were removed at intervals and examined for mother sporocysts but no evidence of these was found. Since the tissue cells of the snail are large and young sporocysts very small it was impossible to identify the parasite structures.

In comparing the two groups of snails which were infected it is seen that the mortality in the latter was very low as compared with the former but much higher than in a control group of snails of the same age, indicating that even in the experiment where the snails ate only a few eggs the resulting infections were responsible for the death of some of the snails. The decrease in mortality of the snails in the latter group, however, may not be due solely to the smaller number of eggs that was fed, but may be due to the fact that most of the snails were larger before they were exposed to infection. There is also the chance that the number of miracidia which freed themselves from the eggs during passage through the digestive system was smaller, since it was noted that a smaller proportion of eggs hatched if they were kept several weeks before being fed to snails.

The large number of sporocysts which was found in the snails seems to indicate that the miracidium gives rise to a sporocyst which in turn produces a daughter generation which gives rise to cercariae. In infection experiments the snails that ate a large number of eggs died, while those which ate only a few lived and shed cercariae. Without the assumption of a daughter generation it would be difficult to account for the large number of sporocysts in these snails which live. Considering the conditions under which natural infection takes place and in the light of experiments it is probable that snails in nature rarely eat more than one or two parasite eggs.

The infection of full-grown snails collected in nature was tried without success. Twenty-three snails were used in the experiment. This is not presented as conclusive evidence that adults do not become infected, but it is, no doubt, safe to assume that the percentage of snails in nature which becomes infected after attaining full size is small. It is true that in the experiments some of the full-grown snails became infected but these were known to be very young, and age may make a difference.

Whether snails become infected by the penetration of the miracidium through the gut wall or by penetration from the exterior after passing through the digestive tract is a question which has given much concern. By using one- or two-day old *Gyraulus parvus*, extended studies have not resulted in definite findings. In spite of the fact that these snails seem ideal for such a study many difficulties were encountered, the worst being the failure of the snails to continue to eat eggs after the esophagus and stomach were full. No matter

how they were treated the snails usually discontinueded eating and partially withdrew into their shells so that the aperture of the shell was in contact with the surface of the dish. It was, therefore, impossible to bring active miracidia into contact with the snail for any length of time. Very little evidence was obtained to indicate that the miracidia penetrated from the exterior after being voided with the feces. The miracidia pay no attention to the snail even when thrown against it, but are promptly carried away by the water currents created by the strong cilia on the snail's body. This was true whether whole or crushed snails or only fragments were used. Observations on miracidia in the presence of snails or snail tissue were made on covered, uncovered, and hanging drop preparations in a moist chamber.

Only in one snail was there any indication of penetration of the miracidium from the exterior. In this preparation there were numerous miracidia and it appeared as if many of them had suddenly penetrated the foot of the snail. This part of the body was crowded with rounded protuberances covered with cilia. The snail died in a short time after observation began and it appeared that what was observed was initial disintegration of the epithelium. Since miracidia do not have cilia at the posterior end of the body a comparison of the structures on the snail with miracidia was attempted. However, in placing a cover-glass on the slide and transferring it to higher magnification most of the structures were freed and all that were examined proved to be epithelial cells. No direct evidence of penetrating miracidia was observed. This case was different, however, from all other observations which were made on these snails under similar conditions.

When miracidia are first voided from the snail they move through the water very slowly while the cilia on the body beat energetically. The miracidium in swimming elongates and contracts while rotating on its long axis. Soon after the escape of the miracidium the action of the cilia becomes progressively slower and vacuoles usually form in the larger posterior epidermal plates, which mark the beginning of disintegration. Most of the miracidia shortly after being freed from the snail attach themselves by the anterior tip of the body, which is adhesive, to the bottom of the container and remain there until death, usually a period of only a few minutes. In many, but not all, of the miracidia the posterior epidermal plates rise from the body of the larva and the edges seem to roll under to form a somewhat hemispherical structure, but they do not completely separate from the miracidium. A very small papilla-like structure can be observed at the anterior end immediately or soon after the miracidia leave the snail and after being in water this structure on some of the miracidia becomes larger. When it reaches maximum size it is spherical and is quite conspicuous. No matter what the size, it is used for attaching the miracidium to a substratum and seems to be the anterior end of the primitive gut which can be everted in various degrees. Why

the miracidium attaches itself was unsolved until it was noticed that a snail in feeding scraped up several of these attached miracidia. The union is so strong that water currents created by the snail's cilia will not detach them. Seeing the snail eat the miracidia suggested the possibility that this was a method by which they could re-enter the snail to become infective. This also supports, though vaguely, the argument that the miracidium penetrates the gut of the snail.

For further proof of this conclusion, day-old snails were given an opportunity to eat parasite eggs. Those which had taken the most eggs were fixed in Bouin's solution when as determined by the aid of a microscope, several escaped miracidia were visible in the gut of the snail. Some of the snails were stained and serial sections 8 μ thick were made of them. Examination failed to show any miracidia penetrating the gut wall. Manter (1926) sectioned snails after feeding them parasite eggs of *Otodistomum cestoides*, a marine trematode, to get evidence of penetration of the miracidia but secured negative results.

This method of hatching the parasite eggs, while not particularly prevalent in forms that have been described, is probably much more common among trematodes than is suspected. In dealing with forms where difficulty is experienced in hatching the eggs it is advisable to try to feed them to snails. Manter (1926) found that the eggs of a marine trematode, *O. cestoides*, parasitic in the Atlantic skate will hatch in the intestine of a snail, *Littorina litorea*, which devoured them in numbers while other snails would eat few or none of the parasite eggs. Schauinsland (1883) believed that the normal method of snail infection for *Distomum tereticolle* (now *Azygia lucii*) was for the egg to be eaten by a snail. Faust (1929) states that the eggs of *Clonorchis sinensis* hatch and the miracidia penetrate the esophagus when ingested by appropriate molluscan hosts.

Cercariae, from the laboratory-raised and infected snail which was dissected April 26, 1930, as well as from other snails, were used in infection experiments with dragonfly nymphs. Ninety-five cercariae were collected from the dissected snail mentioned above and placed in a vial with two laboratory raised *Sympetrum rubicundulum* nymphs, 2 and 2.5 mm. in length. After they had been together two hours the nymphs were returned to their respective containers. Since it was cold the nymphs took in only a few of the cercariae but movements characteristic of nymphs while being attacked were observed.

The infected nymphs, now each 4 mm. long, were examined May 8, 1930. One had a single encysted metacercaria and the other three, in the wall of the branchial basket. The four cysts in tissue were given to a small green frog on the same day. This frog was given two more encysted metacercariae May 18, 1930, taken from another laboratory-raised nymph infected with cercariae from the same batch of snails.

On June 6, 1930, five *Pneumobites parviplexus* were taken from this frog, four of which were mature and the other nearly mature. The immature fluke was 2 mm. long, the other four had many eggs in the uteri and their lengths were 3.75 mm., 3.5 mm. (2), and 3 mm., respectively. This and other observations made on parasites collected from frogs discussed under the topic, Biology of *P. parviplexus*, indicate that this species usually matures in the frog within a month. Hosts were not numerous enough to obtain a series of maturing forms for a comparative study with *P. medioplexus* as to size at the time of maturity. It seems, however, that members of the two species are about the same size when mature. Irwin who had 16 specimens of the parasite on which she based her description of the species, says that her smallest mature worm was 3.9 mm. in length. Whether there were any immature worms in the collection is not stated.

Since green frogs were not raised in the laboratory from eggs the history of the most important individual frogs which were used in experiments will be related. The frog mentioned in the above paragraph was collected as a tadpole from an acid lake near the Biological Station, July 12, 1929, and was kept in an aquarium and fed on decayed lettuce until metamorphosis. It lived in the laboratory as a frog. When transferred to Ann Arbor it was kept in an aquarium and fed on fruit flies and pieces of earthworm. Once during October and again in November, after a feeding of earthworms, the frog was isolated in a jar until feces were deposited. These were negative for any kind of parasite eggs.

Another green frog tadpole with well-developed hind legs was collected from a small lake near Ann Arbor in September, 1929. It was kept in a battery jar, and fed on decaying lettuce, until it transformed in March, 1930. Then it was fed on fruit flies, earthworms, and occasional pieces of beef. Four and five encysted metacercariae in tissue from laboratory-raised and infected dragonfly nymphs, *S. obtrusum*, were fed to this frog, May 9 and 18, respectively, 1930. The frog, 55 mm. long, was examined on March 6, 1931, and three large mature flukes, respectively, 11, 12, and 13 mm. in length, were found in the lungs, the largest that were taken from experimental frogs. Flukes, 13 mm. in length, have been recovered from naturally-infected frogs, but the majority were 8 or 9 mm. if taken from frogs early in the spring when flukes are usually all mature. The largest fluke in Irwin's collection on which the description of the species is based was 8.48 mm. in length. My data show that this species becomes much larger.

Three other green frogs which transformed in the laboratory were given cysts June 23, 1930. As in previous experiments the dragonfly nymphs from which the metacercariae were taken had been raised from eggs and infected in the laboratory with cercariae which had been in turn produced in snails raised and infected in the laboratory by means of parasite eggs previously secured from flukes from a frog infected in the laboratory the previous sum-

mer.* The above frogs were examined, March 6, 1931, two were negative and one had two flukes, each 7 mm. long. In comparing the two trematodes of this frog with the three discussed in the preceding paragraph, the two, 271 days old, although handled in the same way, were much smaller than the three, not more than 305 days old. The difference in age cannot account for the difference in size, but here as in *Pneumonoeces medioplexus*, the difference is to be accounted for on the basis of a difference in environment for parasites presented by frogs of the same species.

In my estimation the account which has been presented and the preliminary experiments recorded in, the Biology of *P. parviplelexus*, establish without a doubt, the life history of *Pneumobites parviplelexus*. The exceedingly dry summer of 1930 prevented the securing of an adequate supply of host material for a more detailed study of the growth of the flukes in the frogs; however, more or less detailed studies of some phases of this life history were made and these are presented in the following paragraphs.

In order to study the escape of cercariae from the snail, several of the most transparent *Gyraulus parvus* which previously had been infected in the laboratory were isolated in a watch-glass of water on August 3, 1930. When placed under the compound microscope one of the snails was held in position by a teasing needle that had been shortened until the needle and handle together were a little longer than the diameter of the watch-glass. Then the needle point was placed in the center of the whorls of a snail which was near the side of the dish, and the handle was rested on the opposite edge of the vessel. The weight of the needle was sufficient to hold the snail in excellent position for study. Certain organs were visible in the body whorl. Opposite the aperture, the heart could be seen beating, and an air chamber extended from the aperture nearly to the heart, occupying the greater part of the diameter of the whorl. Between the chamber and inner side of the whorl the activity of heavy musculature of the snail was visible. On the peripheral part of this section of the whorl was situated the rather large elongated nida-mental gland coextensive with the air chamber. Between the posterior halves of these two organs there was a large space filled with lymph which was moving rapidly from the aperture toward the coil. The anatomy of the snail could be discerned quite well up to the level of the heart. Here the cercariae could first be seen working their way very rapidly through the tissues by elongating and contracting earthworm-fashion. Apparently the suckers were not used, but this fact was difficult to determine definitely. Cercariae always made their way to this lymph stream and as they slipped out of the tissues into it they began lashing their tails. Then by elongating and contracting

* By using the cercariae produced by the two batches of laboratory-raised and infected snails a total of 16 laboratory-raised nymphs of the two species of *Sympetrum* already mentioned were exposed to cercariae and all became infected.

they attempted to use their suckers to work their way forward against the lymph currents. These organs were not used efficiently, however, and it appeared as if it was difficult for them to fasten themselves to any host tissue. For this no explanation can be given, but while watching them attempt to make headway only to be swept back it was noticed that the cercariae were increasing in numbers. Finally by a combined attack, all elongating and contracting at once, they fought what appeared to be lymph currents. Time and again they approached the anterior end only to be suddenly swept back. After lashing their tails a few seconds the trials were always repeated. When the space was somewhat more than half full of cercariae those which were foremost were able to attach themselves to snail tissue at the anterior border of the lymph space. Now they were seen making their way very rapidly through what seemed to be an elaborate system of anastomosing tunnels which had openings from the tissue at the edge of the mantle folded over the rim of the aperture of the shell. The stylets were not used by the cercariae while they made their way earthworm-fashion through the tunnels. These paths through the mantle, which the cercariae followed were made, apparently, by the first ones that escaped from the snail. As soon as the minutest part of the cercaria was exposed at the edge of the thick, muscular mantle all movements of the larva ceased and it appeared to ooze out like a drop of fat. This, apparently, was caused by the tension created by the muscles in the mantle. For about three seconds after being squeezed out the cercariae were somewhat distorted and motionless and it was at this time that they began their usual swimming movement.

Since dragonfly nymphs in the second instar were found infected in nature such an experiment was tried in the laboratory. A vessel full of ice, containing eggs of *Sympetrum rubicundulum*, was removed from the electric refrigerator and as the ice melted the eggs hatched. After a sufficient number had hatched the remaining eggs were returned to the refrigerator. Two of the nymphs when four hours old were put in a container made from the bottom of a two-dram vial. A little water and 25 cercariae were placed in the container. The nymphs were slightly more than a millimeter in length, and each cercaria was about 150 μ long. When all were in the container there was still sufficient room and even yet the chance was small for the cercariae to be swept into the anal opening by the currents of water which the nymph created in respiratory movements. Therefore, after agitating swimming cercariae with a teasing needle for an hour I had succeeded in directing a single cercaria into the anal opening of one nymph and three into the opening of the other. The one which took the three was limp and moved only slightly when touched. It did not recover and was found dead the next morning. The nymph with the single cercaria was not particularly inconvenienced. The usual responses to penetration of cercariae were noted, but they were not as extreme as in

older nymphs. As soon as the single cercaria entered, the nymph was transferred to a slide in a drop of water by means of a pipette and placed under a compound microscope. In this brief time the parasite had already encysted. The cyst, 59 μ in diameter, was located dorsal to and midway between the two ends of the branchial basket. The growth of the cyst was observed and its diameter recorded every other day until the death of the nymph on May 22, 1930. Metacercariae of definitely known age which could be seen in another small nymph that had been infected for other purposes supplied the measurements of growth from the thirteenth to the twenty-second day. These measurements are recorded in Table V.

TABLE V. SHOWING THE GROWTH OF METACERCARIAE; *Pneumobites parviplexus*

Date of Measurements	Age of Metacercariae in days	Diameter of cyst in microns	Increase in size in microns	Remarks
V-12-1930	0	59		Date of encystment
V-14-1930	2	76	17	
V-16-1930	4	85	9	
V-18-1930	6	98	13	
V-20-1930	8	109	11	
V-22-1930	10	120	11	Nymph died
V- 9-1930	13	156	36	Average of four cysts
V-11-1930	15	187	31	Average of same four cysts
V-13-1930	17	187	0	Average of same four cysts
V-15-1930	19	187	0	Average of same four cysts
V-18-1930	22	187	0	Average of same four cysts

An opportunity was afforded to measure two other newly-formed cysts each of which was 61 μ in diameter. Another cyst three days old had a diameter of 79 μ . Several measurements on 7- and 8-day-old cysts from other experiments were obtained and compared with the ones given above. These agreed in measurements with those in the table.

The size of cysts varies in different nymphs. Nymphs of the same size and infected with cercariae from the same source on the same day may show cysts of very different dimensions. From a group of 11 cysts 30 days old taken from several nymphs the largest was 268 μ in diameter, the smallest 223 μ , and the average for the group 243 μ . The increase in the size is not due to a thickening of the cyst but is caused entirely by the growth of the metacercaria. Under ordinary conditions the metacercariae reach their maximum size in 15 days and become four or five times as large as they are immediately after encystment.

The penetration and encystment of a cercaria was observed under somewhat abnormal conditions and was accomplished in the following way. A nymph about five hours old was placed in a container with cercariae. As soon as the larval worm entered the nymph the latter was transferred with a pair of forceps to a drop of water on a slide. A cover-glass was gently and quickly applied, and its pressure forced the inside of the branchial basket out of the anal opening. The cercaria was fastened by the oral sucker to one of the few lamellae. The ventral sucker was not used and the cercarial body stood almost at a right angle with the surface of the host tissue. Although the oral sucker was attached to the lamella the stylet embedded in the sucker was capable of being used. The stylet was manipulated in such a way as to thrust its tip against the chitin at any angle while movement of the sucker slightly changed the size and shape of its cavity. During this time the tail was partially extended, but a few minutes later it became detached and lived for an hour. The now tailless cercaria spent ten minutes in cutting the hole in the chitin, after which the anterior sucker became long and thin and its anterior part was pushed through the hole in the chitin although the cercaria was not in contact with host tissue except at the oral sucker. After getting the anterior end of the oral sucker into the hole deeper penetration was easily accomplished. By use of the sucker and occasional thrusts of the stylet into the tissue the parasite separated it from the chitin. It continued to move around between the chitin and the underlying tissues, in measuring-worm fashion, for more than an hour. Finally a thin membrane was seen around it. For several seconds the stylet area of the oral sucker was applied to the membrane, the anterior end was then folded ventrally and the back of the larva was applied to the cyst wall as if to spread cyst material. Then the metacercaria moved halfway around the cyst. As this process went on the cyst became thicker indicating that it was being formed by the larva from cystogenous material. The cyst, 60μ in diameter, was formed between the chitin and tissue of the lamella. When finished it was slightly flattened and not of uniform thickness, being 2μ thick on the tissue side and 4μ on the chitin side.

The metacercaria was very active for a brief period in the newly-formed cyst but after 30 minutes its movements began to subside, in 20 minutes more it was inactive, except for an occasional change in position. No doubt, the cercaria was disturbed by the conditions under which it encysted for we know that encystment usually requires only about two minutes.

An instance of a double infection has been observed in a snail from Weinsburg Pool which had a double infection with the stylet cercaria of *P. parviplexus* and a monostome. During the time it was kept in the laboratory only stylet cercariae escaped. The monostome cercariae were large, had large eyespots and for these reasons a number of them could be seen through the shell. A positive identification was made later when the snail was dissected. Accord-

ing to Sewell (1922), Filippi (1839) observed double infections in molluscs. Sewell found 18 cases confined to two species of snails in an examination of about 4,000 snails. He found the two snails to be widely distributed and capable of acting as primary host for the greatest number of trematodes. He also noted that one or both of the parasites involved in the double infections belong to a primitive group of trematodes and he attempted to show that the primitive forms are able to develop in more than one molluscan host. Faust (1917) found several cases of double infections. He wrote; "In several instances the same species of snail from the same collection harbored two or more cercariae. For example, at the Maclay Sloughs both *Cercaria trisolenata* and *C. gracillima* were found in the same host species, *Physa*, in fact in the same individual. This case is paralleled by the record of Cort (1915; 55), where the sporocysts of *Cercaria polyadena* and *C. reflexae* were found within the same liver tissue of *Lymnaea reflexa*." Cort (1915b) obtained the cercariae as well as the sporocysts. Ssinitzin (1911) who examined several thousand snails makes one record which may be interpreted as a double infection. Brown (1926) records six double infections three with fork-tailed and stylet cercariae, one with stylet and echinostome, two involving two species of echinostomes. From these records and the observations cited it appears that double infections are not particularly common.

DESCRIPTION OF LIFE HISTORY STAGES

The similarity between the miracidium, sporocyst, cercaria, and metacercaria of *Pneumonoeces medioplexus* and like stages of *Pneumobites parviplexus* has complicated the description of the life history stages. The minuteness of some larval stages and their fragility have added to the labors of the project. The opacity of cercariae and metacercariae has even prevented a complete description of these stages as was anticipated because it has not been possible to distinguish morphologically between most of the larval stages of these two species. Had it not been for the specificity of first, intermediate, and final hosts this work would have been practically impossible.

Since the life history stages are so much alike the eggs, miracidia, and sporocysts are described together and the apparent differences emphasized where they occur; the cercariae and metacercariae are described separately.

Egg. Cort (1915a, p. 223) under "diagnosis" of *P. medioplexus* stated, "eggs average 0.032 mm. in length and 0.0186 mm. in width." Then in the description (p. 227-228) he stated: "The measurements of over two hundred eggs from several different individuals give a variation in length from 0.022 to 0.029 mm. and a range in width from 0.013 to 0.017 mm. The average length was found to be 0.0255 mm. and the average width 0.015 mm. Stafford gives the size of the eggs in this species as 0.028 mm. by 0.018 mm. while Pratt gives the measurements of the eggs for his *Ostiolium formosum* as 0.039

mm. by 0.017 mm. This measurement proved to be an error since in the type specimen of *O. formosum* which I examined, the size of the eggs was found to fall within the limits for the species as given above. Pratt has since re-measured the eggs for *O. formosum** with the same results. He ascribes his original mistake to a confusion of notes."

It will be noted that in Cort's "diagnosis" the measurements are different from those in his description. However, Cort's average measurements, 0.0255 mm. by 0.015 mm., agree with my findings for 25 eggs. Hence, I am considering these measurements as correct for the eggs of *Pneumonoeces medioplexus*.

Irwin (p. 76) says concerning the eggs of *P. parviplexus*: "The eggs range in size from 0.023 mm. to 0.029 mm. in length and from 0.0156 mm. to 0.019 mm. in width, and they average 0.0254 mm. by 0.017 mm. so that they come nearest in size to those of *Pneumonoeces medioplexus*."

My measurements of 36 eggs are almost identical with those of Irwin. These figures show that the eggs of *P. medioplexus* and *Pneumobites parviplexus* (Irwin), are almost the same as to size and shape. There is also no difference in color.

Miracidium. The miracidium of *Pneumonoeces* apparently has not been studied. Several reasons may be given to account for this lack; the thickness and color of the egg shell, difficulty in hatching, and the small size of the miracidium. Siebold (1837) according to Schauinsland (1883) studied the miracidium of a worm in a closely related genus, *Distomum cylindraceum* Zed., now *Haplometra cylindracea* and found the embryo to be naked. Schauinsland studied the miracidium of the same species while within and also outside of the egg and stated that it was impossible to obtain a clear picture through the egg because of its color. He secured the miracidium by pressure on the egg, found that it was covered with long cilia, and that the ciliated covering which was only loosely attached was in many cases shed in one or more pieces. It seems quite probable that the peculiar way in which this covering was sloughed off was due to an abnormal environment and that, perhaps here as in *Pneumonoeces*, the eggs are eaten by the snail. He also observed a very small gut which partially turned out to form a proboscis after the miracidium escaped from the egg.

In order to secure the miracidia in this study the eggs of both species of fluke were usually fed to *Gyraulus parvus* since, as indicated in a previous section, these snails were so much easier to handle. The most intensive work, forming the basis of the following description, was done on the miracidium of *Pneumonoeces medioplexus*. Very dilute aqueous solutions of brilliant cresyl blue, methylene blue, and neutral red prepared according to the methods of Price (1931) were indispensable in the study of the anatomy of the miracidium.

* *Ostiolum formosum* Pratt is a synonym of *P. medioplexus*.

The miracidium (Pl. XXIII, Figs. 6, 7) of *P. medioplexus* is only 26 μ . long and 17 μ . wide. It is ovate, the anterior end is evenly rounded with a small terminal elevation marking the opening of the primitive gut. The posterior end is rounded, notched asymmetrically due to the fact that the ectodermal plates are uneven in size. The miracidium undergoes changes in shape since it possesses well developed powers of contraction and extension. Almost all of the miracidium is covered by two tiers of ciliated epidermal plates (Pl. XXIII, Fig. 6). The cilia are long anteriorly but become progressively shorter posteriorly and at the posterior end of the miracidium they are very rudimentary and represented by stubs which are barely visible and project from the epidermal plates. The most anterior and largest cilia on the body are 5 μ . long. The posterior three-fourths of the body is covered by three elongated ciliated epidermal plates, one of which is larger than the other two and almost covers the entire posterior end. A pronounced asymmetrical indentation is formed where the three come together near the posterior end. The depressions along the somewhat curved longitudinal suture lines are so slight as to be hardly noticeable. The plates are from 1.2 μ . to 1.5 μ . in thickness. The anterior one-fourth of the body is covered with very thin, short, ciliated plates which begin near the anterior end, leaving the tip exposed and free from cilia. Boundaries between these plates were not visible so that their number could not be determined.

The so-called gut, filled with fine granules undergoing Brownian movement, is sac-like and extends from the anterior end to well beyond the middle of the body. There are 4 nuclei (Pl. XXIII, Fig. 7), each 2.5 μ . in diameter, in the wall of the sac-like portion of the gut. At the anterior end of the gut there is a thickened, muscular portion which is provided with longitudinal structures, which may be grooves or rows of glandular material, that stain deeply with aqueous methylene blue. This organ may be everted partially or completely to form a papilla of varying size. When eversion is complete it is a conspicuous ball-like structure. When the muscular portion is everted the longitudinal structures look granular which suggests that possibly these are of a glandular nature rather than grooves. This conclusion is further strengthened by the fact that no unicellular penetration glands were observed in the miracidium. That these areas are glandular is further indicated when, as already stated in the biology of *Pneumonoeces medioplexus*, the miracidia fasten themselves to a dish. Germ cells (Pl. XXIII, Fig. 7) varying in number from two to four, occupy the posterior end of the body of the miracidium and extend to or somewhat beyond the posterior end of the primitive gut. When stained with dilute aqueous methylene blue the large nuclei, 4 μ . in diameter, usually stain but it is only occasionally that the cytoplasm is visible. It is small in amount and, except in cells which have been stained for quite a long time, it is not evenly distributed, therefore the cells are not constant in shape. Two cells (Pl. XXIII,

Fig. 7) of unknown function, with elongate oval nuclei are placed one on either side of the body just underneath the large ectodermal plates at the level of the posterior end of the gut. The anterior half of the body of the miracidium, surrounding the gut, has the appearance of being vacuolated, possibly due to large vacuolated cells, which do not stain with *intra-vitam* stains. The excretory system consists of two small flame cells (Pl. XXIII, Fig. 7) situated one on either side between the germ cells and ectodermal plates, near the posterior end of the body. The excretory tubules were not seen.

Judging from the work of Price who has made a study of all miracidia which have been described and has given a very complete account of the anatomy of the miracidium of *Schistosomatum douthitti* (Cort), the miracidia of *Pneumonoeces medioplexus* and *Pneumobites parviplexus* are quite different from ones which have been described. They differ from others in their greater simplicity, small number of plates and reduction in the number of rows of plates. The difference in size and thickness of the two tiers of plates as well as the asymmetrical body of the miracidium of these flukes seem to be unusual. A comparison of the miracidium of *P. medioplexus* with that of *Pneumobites parviplexus* shows them to be very similar.

Sporocyst. As indicated in a previous section, the mother sporocyst has not been identified. The sporocysts in the liver are considered to be the daughter generation. The living daughter sporocysts (Pl. XXIV, Figs. 19, 20, 21) of *Pneumonoeces medioplexus* as well as those of *Pneumobites parviplexus* (Pl. XXIV, Figs. 22, 23, 24) are elongate sausage-shaped structures. The largest of the former measures 400 μ long by 80 μ wide while the largest of the latter measure 250 μ by 75 μ . The sporocysts of *P. medioplexus* are never as compactly placed in the snail as are those of *P. parviplexus*. Those of the former are brownish in color while those of the latter are whitish. Not more than five cercariae have been counted in the sporocyst of either species although in very small sporocysts germ balls representing cercariae are more numerous. Young sporocysts of *P. medioplexus* which are oval when small, have been found which were completely filled with large cells (Pl. XXIV, Fig. 18) having a diameter of from 20 μ to 23 μ with small nuclei from 5 μ to 7 μ in diameter. These are germ cells, no doubt, which give rise to cercariae. In larger sporocysts germ balls which are still round when they have a diameter of 45 μ or 50 μ fill the cavity. Larger ones are oval suggesting the formation of the cercarial body. More variation in the development of the cercariae was observed in larger sporocysts, since some contained mature cercariae and germ balls which were still round. The germinal end of the wall of young sporocysts is about twice as thick as the other part. The sporocysts of *Pneumobites parviplexus* are much the same as the ones discussed except for size and color. No birth pore was observed in the sporocyst of either species and no cercaria, although moving around in the sporocyst, was seen to make its escape.

Cercaria of *Pneumonoeces medioplexus*. The cercariae (Pl. XXIII, Fig. 8) of *Pneumonoeces medioplexus* and *Pneumobites parviplexus* belong to the ornate group of xiphidiocercariae which was created by Lühe (1909) to embody all "distome Cercarien mit Bohrstachel, deren schlanker Ruderschwanz einen Flossensaum besitzt." Only a few of these cercariae have been described. Lühe included *Cercaria ornata* La Valette and *C. prima* Ssinitzin in this group. Other members of the group are *C. hemilophura* Cort, *C. indicae* XXIV and XXVIII Sewell (1922), *C. trifurcata* Faust (1919), and *C. longistyla* McCoy (1929). Cort (1915) was of the opinion that the fin-fold on the tail was not a basis for a natural subgroup. Sewell mentioned the brevity of the characterization given by Lühe and in a detailed discussion showed that certain ones in the group are very closely related while others show differences which indicate that they are only remotely related. He, therefore, created a subgroup of the *Cercaria ornatae* for *Cercaria prima* Ssin. and *Cercariae Indicae* XXIV and XXVIII, under the heading "Prima" group and gave an extensive diagnosis consisting of six parts as follows:

(1) Distome cercariae of moderate size in which the acetabulum is smaller than the oral sucker and is situated behind the middle of the body length.

(2) The tail is shorter than the body and is furnished with a dorso-ventral fin-fold in its distal portion: the ventral portion of the fin extends further forward than the dorsal part.

(3) The alimentary canal possesses a prepharynx and a pharynx and the intestinal caeca reach back to a point between the posterior margin of the acetabulum and the posterior end of the body.

(4) Salivary glands are present, and consist of four or five pyriform cells.

(5) The excretory bladder is oval or rectangular and the main excretory canals are dilated in the posterior part of their course and open into the bladder by a common median orifice. The excretory formula appears to be $2 \times 6 \times 1 = 12$ flame cells.

(6) Development occurs in oval or sack-shaped sporocysts.

The number of penetration glands and the excretory formula in the cercariae of *P. medioplexus* and *P. parviplexus* are not known but in other respects they fall into the "prima" subgroup. However, until more is known concerning these cercariae it seems best to keep them in the group as created by Lühe.

The cercaria of *P. medioplexus*, as well as the cercaria of *P. parviplexus*, was very difficult to study because of its fragility and opacity. Knowledge of the few structures which are described come as a consequence of studying each cercaria for one thing only. From the time the cercaria was quiet enough to study until it disintegrated was usually only a few seconds. McCoy had difficulty in tracing the ceca of stylet cercariae which he studied and Cort (1915) could not distinguish stylet glands in some forms described by him. These facts indicate that this group is not particularly favorable for anatomical study. This cercaria apparently is one of the smallest of the stylet group.

Its length, when alive with the tail contracted is about 150μ , one-third to one-fourth of which is tail, depending upon the degree of contraction. When the tail is fully extended it is as long as the body. Cercariae were killed by heating on the slide in water under a cover-glass, and the following average measurements were secured: total length 256μ , body length 171μ , body width 82μ , tail length including fold 89μ , widest part of tail 23μ . The cuticula on the body of the cercaria is very thin, about 1μ , and covered with the beginnings of spines which look like minute papillae. They are most easily seen at the posterior end of the body. The tail has a small tissue connection, in a posterior indentation of the cercaria. When the tail is contracted its margin is usually deeply folded, a broad dorso-ventral fin-fold (Pl. XXIII, Fig. 11), 8.5μ wide at the posterior end, passes around the distal end of the tail and extends anteriorly about to the middle on the ventral side and half as far on the dorsal side. The stylet (Pl. XXIII, Figs. 2, 3) measures 17.5μ by 2.36μ and has a pair of low, rounded, dorso-lateral protuberances and a ventral swollen area near the pointed anterior end, posterior to this the shaft is cylindrical. The stylet is one of the last structures to form in the developing cercaria. It makes its appearance as a thin sliver-like structure, the full length of the stylet, around which is deposited additional stylet forming substance until it is completed. There are a number of stylet (penetration) glands on each side of the ventral sucker, but their precise number has not been determined owing to the opacity of the cercariae. One or two pairs can almost always be seen and their nuclei, 4.1μ in diameter, are quite conspicuous. With the death of the cercariae as many as six pairs have been distinguished but this is not the full number. Since some are quite easily seen and others are not, there seems to be a possibility of two kinds of glands. The exceedingly fine ducts have been observed in a few specimens, but their posterior connections with the gland cells have not been seen. These ducts are so fine and so close together that they appear as a single duct on each side. They open well in front of the shoulders of the stylet. The oral and ventral suckers in the living specimen are circular in outline and measure 39μ and 24μ , respectively. The ventral sucker is located about two-thirds of the distance from the anterior end of the body. The thickness of the ventral sucker is slightly greater than half the thickness of the cercaria. There is a short prepharynx, but usually in the inactive cercaria the oral sucker and pharynx are in contact and the prepharynx cannot be seen. When the cercaria elongates the prepharynx is as long as the pharynx. The pharynx in the living cercaria is ovate or pear-shaped and becomes round when the cercaria dies, at which time the pharynx is 19μ in diameter. The esophagus continues for a distance equal to the pharynx, then bifurcates and the ceca, no wider than the pharynx, extend nearly to the posterior end of the body. The ceca have been seen in only two specimens. Cystogenous glands fill the anterior third of the body extending well back of the pharynx. The

fundament of the reproductive organs consists of a finely granular mass, smaller than, and at the level of, the ventral sucker and overlapping its anterior part. The bladder is thin-walled and somewhat rounded when distended but when empty it is irregular and the wall is crenated. A narrow short canal communicates with the excretory pore which is median, on the inner wall of the posterior indentation of the cercarial body and ventral to the tail. Two elongated vesicular cornua which are never voluminous and conspicuous in fresh specimens empty into the bladder. They are separated from the median bladder by very short narrow passages. Each cornu is directed laterally and somewhat anteriorly; at its anterior end each constricts to form a fine duct which has a zig-zag course anteriorly and has been traced as far as the level of the oral sucker. Flame cells have never been seen.

Cercaria of *Pneumobites parvipleus*. This cercaria is almost identical with the one described. A series of measurements shows a slight difference in their size, the cercariae of *P. parvipleus* being slightly smaller. When alive, with the tail contracted, this larva measures 140 μ . With the heat method of killing the following average measurements on a series of cercariae were secured; total length 238 μ , body length 136 μ , body width 70 μ , tail length including fold 89 μ , widest part of tail 20 μ . In comparing average measurements of structures in the living cercariae of the two forms other slight differences were found. The diameter of the oral sucker is 33.9 μ and that of the pharynx is 13.5 μ , both of these being slightly smaller than in *Pneumonoeces mediopleus*. The stylet is somewhat longer, measuring 19.9 μ . The fin-fold in *P. parvipleus* seems to be slightly wider. There may be only five pairs of stylet glands but this could not be definitely determined.

Metacercaria of *Pneumonoeces mediopleus*. The metacercaria (Pl. XXIII, Fig. 14) of *P. mediopleus* is large as compared with the cercaria. For about three days after the encystment of the cercaria it remains a granular vacuolated mass. On the third day the refractile granules, which make the excretory system so conspicuous in the metacercaria, begin to form. On the fourth day the excretory vesicle is filled but not as compactly as it is when the metacercaria becomes older. By this time the vacuolated condition of the body, of the metacercaria has disappeared.

The stylet, instead of being discarded after encystment of the cercaria as usually happens, is retained. It is partially, if not completely, resorbed. The cylindrical shaft is the first part which disappears leaving a structure shaped like an arrow head (Pl. XXIII, Fig. 4), the point of which represents the tip of the stylet. The complete stylet is still present on the third day after encystment, but by the seventh only the arrow-head-shaped remnant remains which is from 3 μ to 5 μ long. On the tenth day nothing remains of the stylet in the metacercaria. As long as a remnant of the stylet is present it can be moved and turned in almost any direction by the metacercaria but it is never

used. The fragment of the stylet, after the shaft has been resorbed, has been observed loose in cysts so that it apparently is discarded. Magath (1917) observed that in the cercaria of *Lissorhis fairporti* the stylet remained in place when the cercaria encysted but he did not discuss its further fate.

The largest cysts observed in experimental infections were 192μ in diameter. The largest living metacercariae when fully extended in water were 710μ long. Fixed specimens of all ages in moderate contraction vary in length from 160μ to 360μ . The average length and width of a series of 22 fixed specimens 32 days old are 292μ by 100μ . Before fixing these living metacercariae they were placed in cold water long enough to stop their movements. When these were compared with a series collected from dragonflies taken in nature where the two species, discussed in this paper, were common in green and leopard frogs it was found that those in nature averaged 100μ by 60μ larger. The metacercariae are elongated and rather narrow. Their thickness is about two-thirds of the width. The anterior end is bluntly rounded and the posterior end somewhat attenuated with a pronounced posterior indentation.

The metacercaria is covered by a cuticula of uniform thickness except for the anterior and posterior ends where it is somewhat thinner. The average thickness of the cuticula is 4μ . In living specimens in water the cuticula swells and becomes thicker. Fundaments of retrorse spines cover the entire body and the degree of development varies considerably with individual specimens. The developing spines extend through the thickness of the cuticle but in only a few of the larvae are the tips of the spines exposed. Both suckers are well developed; the oral is larger than the ventral and the ratio of their sizes is 8:5. In living specimens they are relatively larger than in the preserved ones; although the ratio is invariably the same. The following average measurements of living material were secured while the metacercariae were under some pressure of a cover-glass on the slide and just before disintegration. The average diameter of the oral sucker in living specimens is 81μ , in preserved 56μ , for the ventral sucker in the living specimen 49μ , for preserved 36μ . The most conspicuous structure in the metacercaria is the large "Y" shaped excretory vesicle (Pl. XXIII, Fig. 14) filled with small refractile granules. These granules are uniform in size, averaging 4μ in diameter, and their surface looks rough as if pieces had been chipped out. The metacercariae in water are more or less transparent but the excretory vesicle is made white and conspicuous by the granules. Occasionally when the larvae are first placed in water they become vigorously active and some of the granules are discharged in spurts from the excretory pore. The cornua of the vesicle, two or three times the length of the stem, originate just posterior to the ventral sucker and, in preserved material, extend anteriorly to the posterior end of the pharynx. In living specimens they project somewhat beyond this level and the ends are directed dorso-anteriad to accommodate the pharynx. Occasionally one cornu

is longer than the other. The cornua, as well as the stem, increase in depth, dorso-ventrally, toward their extremities. Posteriorly the stem communicates with a short duct which opens into the posterior indentation of the metacercaria.

Along the sides of the body there are complicated systems of fine tubules extending anteriorly from the posterior end to the level of the oral sucker. Flame cells have not been seen in this species. The digestive system consists of a mouth which opens into a rather narrow, short prepharynx about one-half the length of the pharynx. The lining of the pharynx in the living specimen appears to be spiraled caused by muscles (Pl. XXIII, Fig. 1). The narrow esophagus, which gradually increases in size posteriorly is 1 to 1.5 times as long as the pharynx which opens into it. In some specimens the esophagus is straight, in others it bends to the left or right. In preserved specimens these parts are not clearly visible unless the worms are extended, because the oral sucker and the pharynx are in contact and the longitudinal axis of the latter is turned so as to be at a right angle, usually, with the dorso-ventral surfaces of the metacercaria. The ceca, which have thick walls and a narrow lumen, extend nearly to the posterior end of the body. In their course posteriorly they remain rather close together in front of the ventral sucker, diverge and cross the excretory cornua dorsally at the level of the ventral sucker and then proceed posteriorly, parallel and close to the sides of the worm. The ceca are half the width of the pharynx. Between the intestinal ceca, extending from the bifurcation of the esophagus to the anterior border of the ventral sucker, there is a mass of glands of unknown function, which are yellowish under low power of the microscope. They have about the same position as the penetration glands in the cercaria. The nuclei of these cells have about the same diameter and the appearance of being very much like those of the cercaria, and in both the cercaria and the metacercaria they are very granular. For these reasons it seems possible that these cells in the metacercaria have their origin in the cells of the cercaria. These cells in the metacercaria are ovate in shape, closely massed together and more numerous probably than in the cercaria. As many as 24 nuclei have been counted in each group in the metacercaria. Besides being difficult to see the paired masses are so close together that often they appear to form a single mass. Fine ducts twisted together have been seen lateral to and at the tip of the oral sucker but their connections with the cells posteriorly have never been discerned. One explanation seems possible for the presence of these glands in the metacercariae. Since these larvae feed on blood they must work their way at least through epithelial and connective tissues and since their spines are not yet developed for anchoring the body in the tissues of the host these glands possibly secrete a solvent which aids the larva in securing an immediate food supply by dissolving or softening the tissues. As already mentioned, in the subject of the biology of *Pneumonoeces*

medioplexus, some of the larvae have a digestive system full of blood before they even reach the glottis. Two irregular masses side by side and dorsal to the ventral sucker represent the fundaments of the ovary and seminal receptacle. The rudiments of testes are near the posterior end of the metacercaria, and are to the inside of the ceca, near their posterior ends. The testes have an oblique arrangement as in the adult, but cannot be seen in all living specimens. Stained, fixed material shows that they are very small and for this reason, they possibly are overlooked in the living specimen.

Metacercaria of *Pneumobites parviplexus*. The metacercaria (Pl. XXIII, Figs. 12, 13) of *P. parviplexus* is very much like the one described and only a few differences have been observed. These metacercariae become somewhat larger than those of *P. medioplexus*. The average length and width of a series of fixed specimens 28 days old are 410μ by 130μ , the average diameter of the oral sucker is 59μ , ventral 39μ , and the ratio of the oral to the ventral sucker in both living and fixed specimens 6:4. The fundaments of the testes, oval in shape, are larger and better developed than in the other species. More variation in the size of the excretory granules exists in the metacercariae of this species but the average size is the same.

The number of gland cells anterior to the ventral sucker may be less in this form, since only 12 nuclei could be counted on a side. Three exceedingly small flame cells were seen in one 32-day-old metacercaria. The position of the flame cells is shown in Plate XXIII, Fig. 12.

SUMMARY AND CONCLUSIONS

The life histories of *Pneumonoeces medioplexus* Stafford and *Pneumobites parviplexus* (Irwin) have been presented, the first to be worked out in these two genera.

Determination of the life cycle of *P. medioplexus* is based on preliminary experiments and a final experiment in which the hosts, *Planorbula armigera* (Say), *Sympetrum obtrusum* (Hagen) and *S. rubicundulum* (Say), and *Rane pipiens* Schreber which had been raised from eggs were infected in the laboratory.

Wood frogs cannot be infected experimentally with metacercariae of *P. medioplexus*, and no natural infections have been observed in this species.

Dragonfly nymphs of the genus *Leucorrhinia* cannot be infected with the cercariae of *Pneumonoeces medioplexus*, although they are morphologically indistinguishable from nymphs of *Sympetrum*.

The determination of the life cycle of *Pneumobites parviplexus* (Irwin) is based on preliminary experiments and a final experiment in which the hosts *Gyraulax parvus* (Say), *Sympetrum obtrusum* (Hagen) and *S. rubicundulum* (Say) which had been raised from eggs were infected in the laboratory. *Rana clamitans* Latr., the final host, was raised from trematode-free tadpoles and infected in the laboratory.

The eggs of both species of lung fluke hatch when they are passed through the digestive system of snails. Other snails than the host species also activate the miracidia, causing the eggs to hatch, without, however, becoming infected.

The production of free swimming cercariae of *Pneumobites parviplexus* in *Gyraulus parvus* occurs in experimental infections as early as the thirty-second day and experiments with *Pneumonoeces medioplexus* indicate that the period of time necessary to produce infections in *Planorbula armigera* is somewhat longer.

When the snails were given too many parasite eggs, they were killed before mature cercariae developed.

Cercariae of *P. medioplexus* escape from the snail chiefly during the night while those of *Pneumobites parviplexus* make their escape during the afternoon.

Cercariae of both species enter the dragonfly nymph passively by being drawn into the rectal respiratory chamber by means of the respiratory currents, and penetrate into the tissues of the branchial basket, where they encyst.

Except for the first instar which is of very short duration, dragonfly nymphs are capable of being infected throughout their entire larval life.

The encysted cercaria retains its stylet which degenerates in place, thus preventing the possibility of the identification of a metacercaria by means of a discarded stylet.

Metacercariae may attain maximum size in the dragonfly nymph in 14 or 15 days.

As many as 220 encysted lung fluke metacercariae have been found in a single naturally infected adult *Sympetrum*.

The average time required for either species to mature in the frog is a month.

Frogs cannot be killed by overdosing them with metacercariae, since the frogs discard them until a comparatively small number of parasites remain. The largest number of mature parasites (74) in a natural infection was taken from a leopard frog and these were all *Pneumonoeces medioplexus*.

Size of mature flukes is independent of the size of the host and the number of flukes present, but depends, apparently, upon the physiological condition of the host.

Frogs annually lose their old infection and pick up a new one.

Tadpoles cannot be infected experimentally with metacercariae and no naturally infected tadpoles have been found.

The life cycle of *P. medioplexus* can be completed in two months under controlled conditions, while that of *P. parviplexus* requires a somewhat longer time.

The miracidium, daughter sporocyst, cercaria and metacercaria, respectively

of the two species are extraordinarily similar. In fact, of these larval stages only the metacercariae can be distinguished from each other.

The larval forms of these two species occur in closely related genera of snails and in dragonflies of the same genus. It is possible, however, that other species of lung flukes make use of quite different hosts than these.

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EXPLANATION OF PLATES XXIII AND XXIV

Figures 4, 15, and all others accompanied by a scale were made with the aid of the camera lucida.

Abbreviations

- a anterior epidermal plate
- d ducts of penetration glands
- e excretory bladder
- f flame cell
- g germ cell
- h gland cells of unknown function
- n nucleus in the gut wall
- p posterior epidermal plate
- r fundament of reproductive organs
- s stylet remnant
- v nucleus of penetration gland

PLATE XXIII

- FIG. 1. Pharynx, metacercaria of *Pneumonoeces medioplexus*.
FIG. 2. Stylet, dorsal view, of *Pneumonoeces medioplexus*.
FIG. 3. Stylet, lateral view, of *Pneumonoeces medioplexus*.
FIG. 4. Stylet remnant from a seven-day-old metacercaria of *Pneumobites parviplexus*.
FIG. 5. Lateral view of the stylet remnant of *P. parviplexus* in the normal position. Drawn from a living specimen.
FIG. 6. Miracidium, *Pneumonoeces medioplexus*, showing plates and cilia.
FIG. 7. Miracidium, *P. medioplexus*, showing internal anatomy.
FIG. 8. Cercaria, *Pneumonoeces medioplexus*.
FIG. 9. Cercaria, *Pneumobites parviplexus*, lateral view. Drawn from a mounted specimen, killed in hot formalin.
FIG. 10. Tail of cercaria of *P. parviplexus*, lateral view, from a mounted specimen killed in hot formalin.
FIG. 11. Tail of cercaria of *Pneumonoeces medioplexus*, lateral view, from a mounted specimen killed in hot formalin.
FIG. 12. Large metacercaria of *P. parviplexus*, dorsal view, 21 days old.
FIG. 13. Small metacercaria of *P. parviplexus*, ventral view, seven days old.
FIG. 14. Metacercaria of *P. medioplexus*, dorsal view, ten days old.
FIG. 15. Section of the body wall of a medium sized daughter sporocyst.
FIG. 16. *Sympetrum* nymph.
FIG. 17. Encysted metacercaria in a gill lamella.



PLATE XXIV

- FIG. 18. Germ balls and two germ cells from a daughter sporocyst of *P. medioplexus*.
FIGS. 19, 20. Daughter sporocysts of *P. medioplexus*, the triradiate one is unusual.
FIG. 21. Young daughter sporocyst of *P. medioplexus* full of germ balls.
FIGS. 22, 24. Young daughter sporocyst of *P. parvioplexus*.
FIG. 23. Young daughter sporocyst of *P. parvioplexus*, subject to some pressure, showing thickness of its wall.
FIG. 25. Shell of *Planorbula armigera*.
FIG. 26. Shell of *P. armigera*, side view.
FIG. 27. Shell of *Gyraulus parvus*.
FIG. 28. Shell of *G. parvus*, side view.

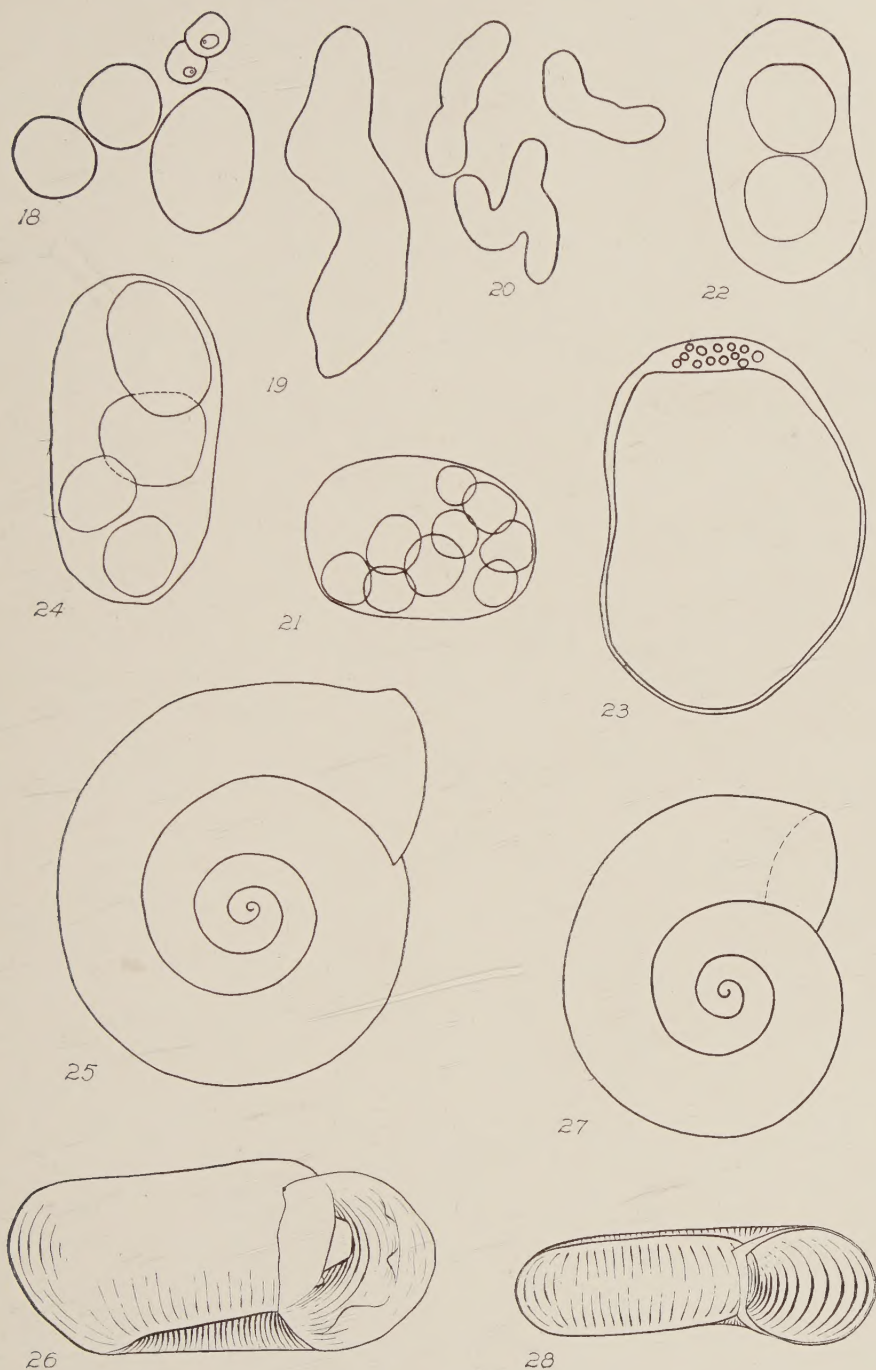


PLATE XXIV

